Dual Nicotinic Acetylcholine Receptor 42 Antagonists/7 Agonists: Synthesis, Docking Studies, and Pharmacological Evaluation of Tetrahydroisoquinolines and Tetrahydroisoquinolinium Salts

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Dual Nicotinic Acetylcholine Receptors α4β2 Antagonists/α7 Agonists: Synthesis, Docking Studies and Pharmacological Evaluation of Tetrahydroisoquinolines and Tetrahydroisoquinolinium Salts

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1 Materials, methods and abbreviations (chemistry)

Materials. Reagents were obtained from commercial suppliers and used without further purifications. Syringes which were used to transfer anhydrous solvents or reagents were purged with nitrogen prior to use. Other solvents were analytical or HPLC grade and were used as received. Compounds **1**, **3a**–**f**, **5a**–**g**, **8a**–**c** and **9** were prepared according to either known or slightly modified literature procedures [1–5, 12]. Compound **3d** (CAS number 20412-65-1) which is also known as *N*-methyltetrahydropapaverine, is commercially available.

Methods. Yields refer to isolated compounds estimated to be > 95 % pure as determined by HPLC and LC-MS. Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} plates from Merck (Germany). Visualization was accomplished under UV lamp (254 nm). Flash column chromatography was performed on chromatography grade, silica gel 60 Å particle size 35–70 micron from Fisher Scientific using the solvent system as stated. Microwave-assisted synthesis was carried out in a Biotage Initiator apparatus operating in single mode; the microwave cavity producing controlled irradiation at 2.45 GHz (Biotage AB, Uppsala, Sweden). The reactions were run in sealed vessels. These experiments were performed by employing magnetic stirring and a fixed hold time using variable power to reach (during 1–2 min) and then maintain the desired temperature in the vessel for the programmed time period. The temperature was monitored by an IR sensor focused on a point on the reactor vial glass. The IR sensor was calibrated to internal solution reaction temperature by the manufacturer. ^{1}H and ^{13}C NMR spectra were recorded on Varian 300 (Mercury and Gemini instruments) or on Brucer (400 and 600 MHz) instruments using $CDCl_3$ or DMSO-d_6 as deuterated solvents and with the residual solvent as the internal reference. For all NMR experiments the deuterated solvent signal was used as the internal lock. Coupling constants (*J* values) are given in Hertz (Hz). Multiplicities of ^{1}H NMR signals are reported as follows: s, singlet; d, doublet; dd, doublet of doublets; dt, doublet of triplets; t, triplet; q, quartet; m, multiplet; br, broad signal. Optical rotations were measured as $[\alpha]_D$ values with a Bellingham and Stanley model ADP410 polarimeter (measurements made at the sodium D-line). Melting points (mp) were determined using an MPA100 Optimelt melting point apparatus and are uncorrected. Low-resolution mass spectra (LRMS) were obtained by LC/MS (Agilent 1100, Xbridge column, ESI detector). High-resolution mass spectra (HRMS) were obtained using a Bruker Daltonics MicroTOF instrument. Preparative HPLC separations were performed on a Daciel Chiralpak® AD-H column (250 x 20mm; 5 µm) and eluted at 20 mL/min with heptane–isopropanol–diethylamine (95:5:0.1) for compound **5c** and heptane–isopropanol–diethylamine (90:10:0.1) for compound **5d** as eluent during 15 min at 25 °C. The column was connected to a Dionex HPLC system consisting of an UltiMate 3000 pump, a TSP ASI3000 Automated Sample Injector, and an UltiMate 3000 photodiode array detector (210 nm). For HPLC control, data collection and data handling, Chromeleon software v. 6.80 was used. Analytical HPLC experiments were performed on a Lux® Amylose-1 column (250 × 4.6 mm ID; 3 µm) Phenomenex equipped with a Lux® Amylose-1 guard column (4 × 3.0 mm ID) and eluted at 1.0 mL/min with heptane–isopropanol–diethylamine (95:5:0.1) as eluent during 20 min at 25 °C. The column was connected to a Dionex HPLC system consisting of an UltiMate 3000 pump, a TSP ASI3000 Automated Sample Injector, and an UltiMate

3000 photodiode array detector (210 nm). For HPLC control, data collection and data handling, Chromeleon software v. 6.80 was used.

Abbreviations. The following abbreviations are used: HPLC: high-performance liquid chromatography; MW: microwave; DMF: *N*,*N*-dimethylformamide; DCM: dichloromethane; THF: tetrahydrofuran; LAH: lithium aluminium hydride; TfOH: triflic acid; AcOH: acetic acid; MeOH: methanol; TEA: triethylamine; Et_2O: diethyl ether; EtOAc: ethyl acetate; EtOH: ethanol; ee: enantiomeric excess.

2 Synthetic procedures

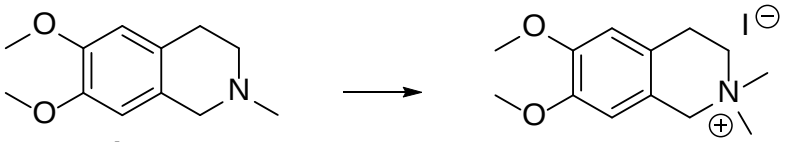
General procedure 1: synthesis of isoquinolinium salts 2a–e, 4a–f and 6a–g.

To a solution of the chosen isoquinoline derivative **1**, **3a–f** or **5a–g** (1 equiv) dissolved in dry acetone (typically 0.3–0.4 M solution) was added the appropriate alkyl or benzyl halide (10 equiv) at room temperature. The mixture was stirred at this temperature for about 12 h in the dark then filtered. The resulting solid was washed with dry acetone to afford the desired pure isoquinolinium salt **2a**–**e**, **4a**–**f** or **6a**–**g**.

General procedure 2: synthesis of isoquinolinium salts (*R*)-6c, (*S*)-6c, (*R*)-6d and (*S*)-6d.

To a solution of the chosen enantiopure isoquinoline derivative (*R*)-**5c**, (*S*)-**5c**, (*R*)-**5d** or (*S*)-**5d** (1 equiv) dissolved in dry acetone (typically 0.3–0.4 M solution) was added methyl iodide (15 equiv) at room temperature. The mixture was stirred at this temperature for about 2 h in the dark then filtered. The resulting solid was washed with dry acetone to lead to pure isoquinolinium salt (*R*)-**6c**, (*S*)-**6c**, (*R*)-**6d** or (*S*)-**6d**.

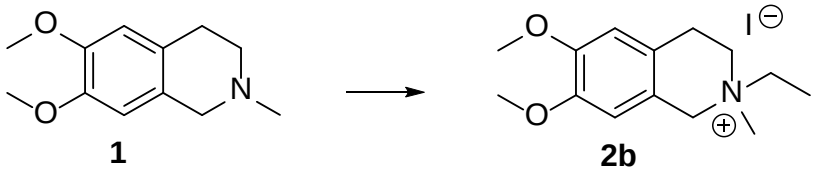
6,7-Dimethoxy-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolin-2-ium iodide 2a [6]



1 **2a**

Starting from tetrahydroisoquinoline **1** (207 mg, 1 mmol) and following general procedure 1, tetrahydroisoquinolinium salt **2a** was obtained as a white solid (335 mg, 96%); dec 240 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.86 (s, 1H), 6.73 (s, 1H), 4.46 (s, 2H), 3.74 (s, 3H), 3.72 (s, 3H), 3.58–3.67 (m, 2H), 3.12 (s, 6H), 3.00–3.09 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 149.3, 148.6, 121.9, 119.3, 112.3, 110.5, 62.9, 59.2, 56.4, 56.3, 51.3 (2C), 23.9; HRMS (APPI): M^+ found 222.1490. $C_{13}H_{20}NO_2$ requires 222.1492.

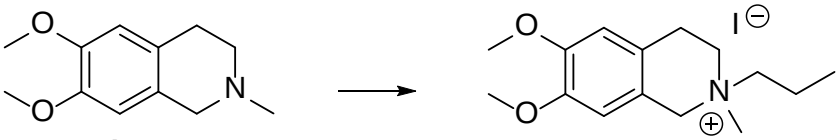
6,7-Dimethoxy-2-ethyl-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium iodide 2b



1 **2b**

Starting from tetrahydroisoquinoline **1** (194 mg, 0.94 mmol) and following general procedure 1, tetrahydroisoquinolinium salt **2b** was obtained as a white solid (277 mg, 81%); mp 207–209 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.89 (s, 1H), 6.78 (s, 1H), 4.42–4.51 (m, 2H), 3.76 (s, 3H), 3.74 (s, 3H), 3.55–3.72 (m, 2H), 3.42 (q, J = 7.2, 2H), 2.98–3.09 (m, 5H), 1.33 (t, J = 7.2, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 148.6, 147.9, 121.7, 118.5, 111.6, 110.0, 60.3, 57.9, 56.4, 55.61, 55.58, 46.0, 22.8, 7.4; HRMS (APPI): M^+ found 236.1649. $C_{14}H_{22}NO_2$ requires 236.1651.

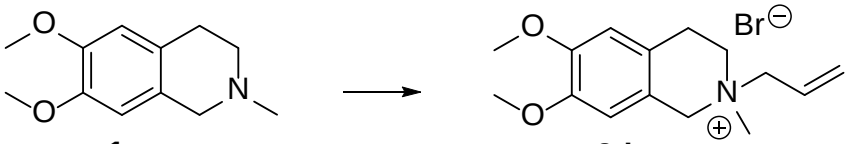
6,7-Dimethoxy-2-methyl-2-propyl-1,2,3,4-tetrahydroisoquinolin-2-ium iodide 2c



1 **2c**

Starting from tetrahydroisoquinoline **1** (143 mg, 0.69 mmol) and following general procedure 1, tetrahydroisoquinolinium salt **2c** was obtained as a white solid (235 mg, 90%); mp 220–223 °C; ^{1}H NMR (600 MHz, DMSO-d_6): 6.88 (s, 1H), 6.77 (s, 1H), 4.49 (d, J = 14.8, 1H), 4.45 (d, J = 14.8, 1H), 3.76 (s, 3H), 3.73 (s, 3H), 3.58–3.69 (m, 2H), 3.26–3.34 (m, 2H, partially under the solvent peak), 3.01–3.08 (m, 5H), 1.74–1.87 (m, 2H), 0.92 (t, J = 7.2, 3H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 148.6, 147.9, 121.7, 118.5, 111.6, 109.9, 63.8, 60.8, 56.9, 55.61, 55.56, 46.7, 22.9, 15.0, 10.6; HRMS (APPI): M^+ found 250.1805. $C_{15}H_{24}NO_2$ requires 250.1802.

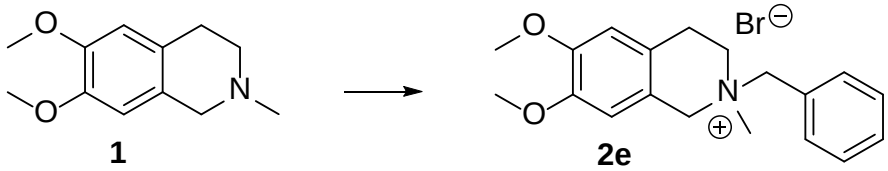
2-Allyl-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium bromide 2d



1 **2d**

Starting from tetrahydroisoquinoline **1** (108 mg, 0.52 mmol) and following general procedure 1, tetrahydroisoquinolinium salt **2d** was obtained as a white solid (130 mg, 76%); mp 183–185 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 6.89 (s, 1H), 6.79 (s, 1H), 6.11–6.20 (m, 1H), 5.63–5.69 (m, 2H), 4.52 (d, J = 14.8, 1H), 4.44 (d, J = 14.8, 1H), 4.05–4.12 (m, 2H), 3.76 (s, 3H), 3.73 (s, 3H), 3.66–3.71 (m, 1H), 3.59–3.65 (m, 1H), 3.07 (t, J = 6.0, 2H), 3.03 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 148.7, 147.9, 127.9, 125.5, 121.6, 118.4, 111.6, 110.0, 64.5, 60.3, 56.7, 55.61, 55.56, 46.6, 22.7; HRMS (APPI): M^+ found 248.1650. $C_{15}H_{22}NO_2$ requires 248.1651.

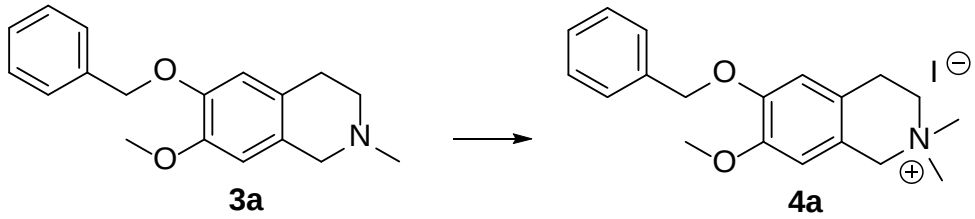
2-Benzyl-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-ium bromide 2e



1 **2e**

Starting from tetrahydroisoquinoline **1** (114 mg, 0.55 mmol) and following general procedure 1, tetrahydroisoquinolinium salt **2e** was obtained as a white solid (139 mg, 67%); dec 232 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.52–7.62 (m, 5H), 6.90 (s, 1H), 6.81 (s, 1H), 4.73 (d, J = 12.6, 1H), 4.70 (d, J = 12.6, 1H), 4.62 (d, J = 14.8, 1H), 4.29 (d, J = 14.8, 1H), 3.68–3.82 (m, 8H), 3.07–3.19 (m, 2H), 2.94 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 148.7, 148.0, 133.1 (2C), 130.3, 129.0 (2C), 127.5, 121.7, 118.3, 111.6, 110.1, 66.2, 59.8, 57.1, 55.61, 55.56, 45.3, 22.8; HRMS (APPI): M^+ found 298.1809. $C_{19}H_{24}NO_2$ requires 298.1807.

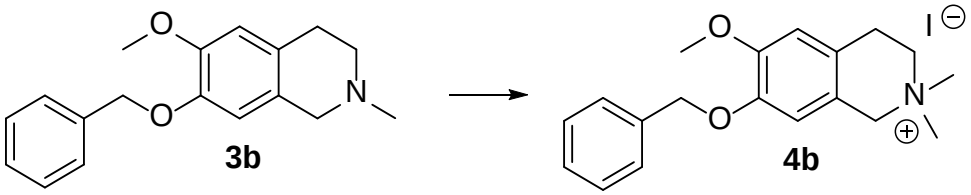
6-Benzyloxy-2,2-dimethyl-7-methoxy-1,2,3,4-tetrahydroisoquinolin-2-ium iodide 4a



3a **4a**

Starting from tetrahydroisoquinoline **3a** (170 mg, 0.60 mmol) and following general procedure 1, tetrahydroisoquinolinium salt **4a** was obtained as an off-white solid (180 mg, 71%); mp 178–180 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.27–7.46 (m, 5H), 6.99 (s, 1H), 6.77 (s, 1H), 5.06 (s, 2H), 4.48 (s, 2H), 3.74 (s, 3H), 3.58–3.67 (m, 2H), 3.13 (s, 6H), 2.99–3.08 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 148.9, 148.3, 137.4, 129.1 (2C), 128.6, 128.5 (2C), 121.9, 119.8, 113.9, 110.8, 70.7, 62.9, 59.2, 56.5, 51.4 (2C), 23.9; HRMS (APPI): M^+ found 298.1801. $C_{19}H_{24}NO_2$ requires 298.1804.

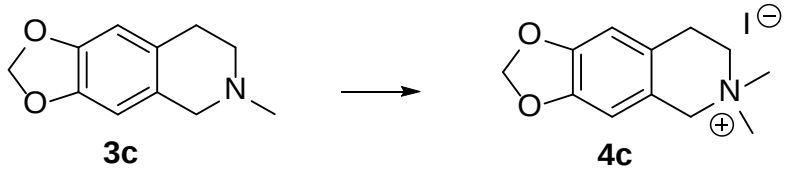
7-Benzyloxy-2,2-dimethyl-6-methoxy-1,2,3,4-tetrahydroisoquinolin-2-ium iodide 4b



3b **4b**

Starting from tetrahydroisoquinoline **3b** (170 mg, 0.60 mmol) and following general procedure 1, tetrahydroisoquinolinium salt **4b** was obtained as a pale yellow solid (192 mg, 74%); dec 180 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27–7.45 (m, 5H), 6.71 (s, 1H), 6.65 (s, 1H), 5.09 (s, 2H), 4.67 (s, 2H), 3.96–4.04 (m, 2H), 3.87 (s, 3H), 3.56 (s, 6H), 3.10–3.20 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.5, 148.1, 136.5, 128.7 (2C), 128.3, 127.6 (2C), 121.0, 117.5, 112.1, 111.8, 71.5, 64.4, 60.3, 56.5, 52.2 (2C), 24.2; HRMS (APPI): M^+ found 298.1803. $C_{19}H_{24}NO_2$ requires 298.1804.

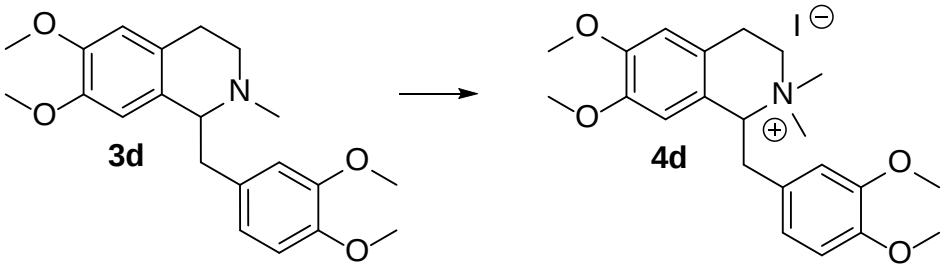
6,7-Methylenedioxy-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolin-2-ium iodide 4c



3c **4c**

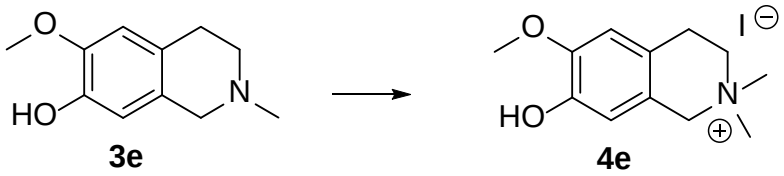
Starting from tetrahydroisoquinoline **3c** (191 mg, 1 mmol) and following general procedure 1, tetrahydroisoquinolinium salt **4c** was obtained as a white solid (297 mg, 96%); dec 216 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.85 (s, 1H), 6.75 (s, 1H), 6.00 (s, 2H), 4.45 (s, 2H), 3.57–3.68 (m, 2H), 3.12 (s, 6H), 2.98–3.08 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 147.9, 147.1, 123.5, 120.5, 109.1, 107.2, 102.0, 63.0, 59.0, 51.3 (2C), 24.2; HRMS (APPI): M^+ found 206.1176. $C_{12}H_{16}NO_2$ requires 206.1182.

1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolin-2-ium iodide 4d [6]



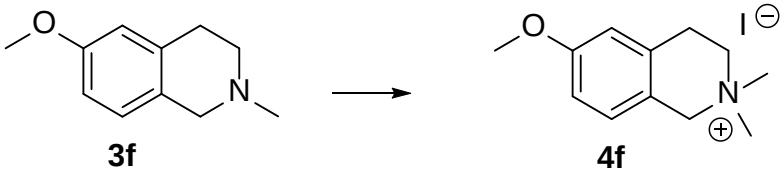
Starting from tetrahydroisoquinoline **3d** (109 mg, 0.30 mmol), which is also known as (±)-laudanosine, and following general procedure 1 (except that the stirring was performed at 35°C instead of room temperature), tetrahydroisoquinolinium salt **4d** (also known as (±)-*N*-methyllaudanosinium iodide) was obtained as a white solid (124 mg, 82%); dec 209 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.89 (d, *J* = 2.0, 1H), 6.69 (d, *J* = 8.1, 1H), 6.63 (s, 1H), 6.46 (dd, *J* = 8.1 and *J* = 2.0, 1H), 5.74 (s, 1H), 5.22 (dd, *J* = 10.4 and *J* = 3.9, 1H), 3.97–4.03 (m, 1H), 3.91 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H), 3.70–3.79 (m, 2H), 3.52 (s, 3H), 3.39 (s, 3H), 3.19–3.27 (m, 1H), 3.07–3.14 (m, 1H), 2.83–2.88 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 149.43, 149.37, 148.5, 147.2, 126.6, 122.8, 121.3, 119.4, 113.6, 111.6, 111.2, 110.6, 72.1, 57.0, 56.0, 55.9, 55.6, 55.3, 52.9, 50.9, 38.1, 23.7. All the other analytical data were consistent with the ones reported in the literature [6].

7-Hydroxy-2,2-dimethyl-6-methoxy-1,2,3,4-tetrahydroisoquinolin-2-ium iodide 4e [7]



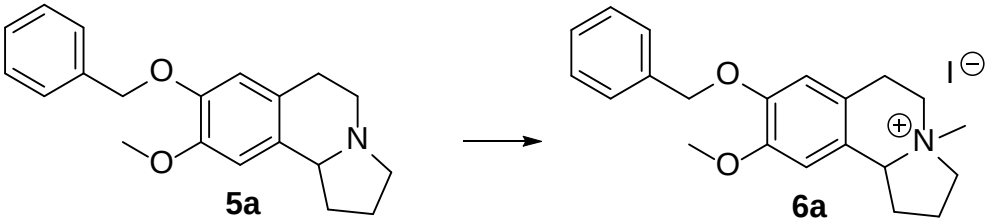
Starting from tetrahydroisoquinoline **3e** (55 mg, 0.28 mmol) and following general procedure 1 (except that the stirring was performed at 35°C instead of room temperature), tetrahydroisoquinolinium salt **4e** was obtained as a white solid (81 mg, 85%); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.13 (s, 1H), 6.84 (s, 1H), 6.54 (s, 1H), 4.42 (br s, 2H), 3.76 (s, 3H), 3.62 (t, *J* = 6.5, 2H), 3.11 (s, 6H), 3.02 (t, *J* = 6.5, 2H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 147.8, 145.7, 119.5, 118.8, 113.1, 112.0, 62.0, 58.5, 55.6 (2C), 50.5, 23.0.

2,2-Dimethyl-6-methoxy-1,2,3,4-tetrahydroisoquinolin-2-ium iodide 4f [4]



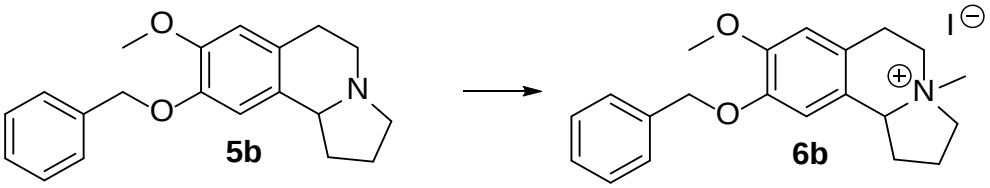
Starting from tetrahydroisoquinoline **3f**[1] (61 mg, 0.34 mmol) and following general procedure 1, tetrahydroisoquinolinium salt **4f** was obtained as a white solid (71 mg, 65%); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.09–7.14 (m, 1H), 6.86–6.92 (m, 2H), 4.52 (s, 2H), 3.76 (s, 3H), 3.66 (t, J = 6.5, 2H), 3.10–3.17 (m, 8H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 158.9, 130.8, 128.1, 119.0, 113.6, 113.2, 60.1, 58.3, 55.2 (2C), 50.5, 24.2.

8-Benzyloxy-9-methoxy-4-methyl-2,3,4,5,6,10b-hexahydro-1*H*-pyrrolo[2,1-*a*]isoquinolin-4-ium iodide 6a



Starting from indolizidine derivative **5a** (60 mg, 0.19 mmol) and following general procedure 1, indolizidinium salt **6a** was obtained as an off-white solid (55 mg, 63%); dec 216 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.44 (d, J = 7.2, 2H), 7.40 (t, J = 7.2, 2H), 7.35 (t, J = 7.2, 1H), 6.99 (s, 1H), 6.83 (s, 1H), 5.07 (s, 2H), 4.63 (br t, J = 9.0, 1H), 3.72–3.82 (m, 5H), 3.59–3.63 (m, 1H), 3.49–3.54 (m, 1H), 3.05–3.13 (m, 4H), 2.91–2.97 (m, 1H), 2.67–2.73 (m, 1H), 2.09–2.17 (m, 2H), 1.92–1.99 (m, 1H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 148.4, 147.6, 136.9, 128.5 (2C), 127.9, 127.8 (2C), 122.6, 120.3, 113.2, 110.5, 71.2, 70.0, 65.6, 55.8, 52.7, 47.5, 31.9, 23.0, 19.6; HRMS (APPI): M^+ found 324.1957. $C_{21}H_{26}NO_2$ requires 324.1958.

9-Benzyloxy-8-methoxy-4-methyl-2,3,4,5,6,10b-hexahydro-1*H*-pyrrolo[2,1-*a*]isoquinolin-4-ium iodide 6b

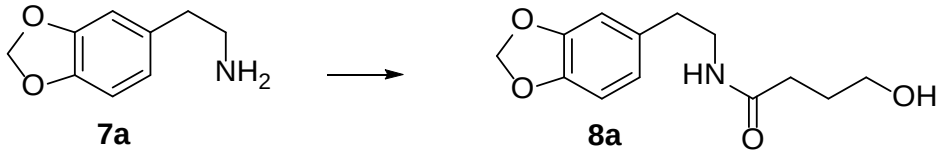


Starting from indolizidine derivative **5b** (98 mg, 0.32 mmol) and following general procedure 1, indolizidinium salt **6b** was obtained as an off-white solid (91 mg, 64%); dec 224 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.44 (d, J = 7.2, 2H), 7.40 (t, J = 7.2, 2H), 7.34 (t, J = 7.2, 1H), 6.92 (s, 1H), 6.91 (s, 1H), 5.08 (d, J = 11.9, 1H), 5.04 (d, J = 11.9, 1H), 4.60 (br t, J = 9.6, 1H), 3.71–3.81 (m, 5H), 3.58–3.66 (m, 1H), 3.50–3.55 (m, 1H), 3.07–3.18 (m, 4H), 2.94–3.01 (m, 1H), 2.61–2.70 (m, 1H), 2.06–2.18 (m, 2H), 1.89–1.97 (m, 1H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 148.9, 147.0,

[1] 6-Methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline **3f**; colorless oil; R_f = 0.56 (EtOAc–MeOH–NH_4OH (28%), 60:10:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.93 (d, J = 8.4, 1H), 6.70 (dd, J = 8.4 and J = 2.7, 1H), 6.64 (d, J = 2.9, 1H), 3.77 (s, 3H), 3.56 (s, 2H), 2.92 (t, J = 6.0, 2H), 2.71 (t, J = 6.0, 1H), 2.48 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 158.2, 134.9, 127.5, 126.5, 113.4, 112.3, 57.4, 55.4, 52.8, 46.0, 29.4.

136.8, 128.4 (2C), 127.92, 127.88 (2C), 122.1, 120.8, 112.0, 111.9, 71.1, 70.1, 65.5, 55.7, 47.4, 40.1, 31.9, 23.0, 19.5; HRMS (APPI): M^+ found 324.1956. $C_{21}H_{26}NO_2$ requires 324.1958.

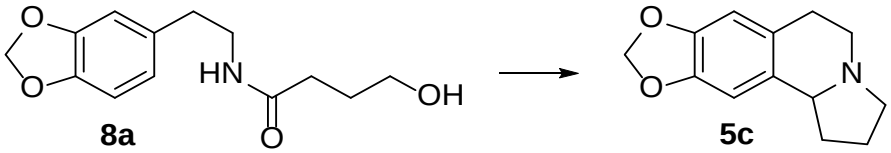
***N*-(3,4-Methylenedioxyphenethyl)-4-hydroxybutanamide 8a** [2]



7a **8a**

To a MW vial were successively added 2-(3,4-methylenedioxyphenyl)ethylamine **7a** (1.57 g, 9.50 mmol, 1 equiv) and *γ*-butyrolactone (0.90 g, 10.45 mmol, 1.1 equiv) at room temperature. The MW vial was sealed and heated under MW conditions for 15 min at 150 °C. The resulting viscous orange oil was purified by column chromatography on silica gel using DCM–MeOH (10:1) as eluent to provide the titled compound **8a** as an off-white solid (1.81 g, 76%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.75 (d, *J* = 7.9, 1H), 6.68 (d, *J* = 1.7, 1H), 6.63 (dd, *J* = 7.9 and *J* = 1.7, 1H), 5.94 (s, 2H), 5.59 (br s, 1H), 3.68 (t, *J* = 5.4, 2H), 3.47 (dt, *J* = 6.9 and *J* = 5.8, 2H), 2.73 (t, *J* = 6.9, 2H), 2.68 (t, *J* = 5.3, 1H), 2.31 (t, *J* = 6.9, 2H), 1.82–1.89 (m, 2H). All the other analytical data were consistent with the ones already reported in the literature [2].

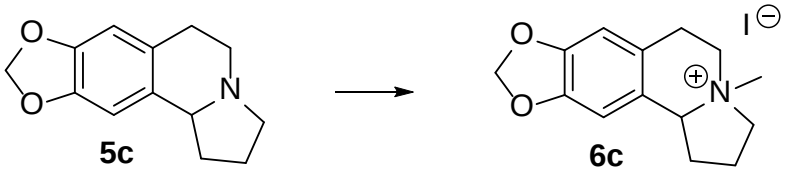
8,9-Methylenedioxy-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline 5c [2]



8a **5c**

To a MW vial were successively added compound **8a** (1.51 g, 6.01 mmol, 1 equiv), acetonitrile (13.2 mL) and phosphorous (III) oxychloride (4.61 g, 30.05 mmol, 5 equiv) at room temperature. The MW vial was sealed and heated under MW conditions for 15 min at 150 °C. Volatiles were removed under reduced pressure and the resulting material was dissolved in an AcOH–MeOH (1:12, 13 mL) mixture prior to addition of sodium borohydride (0.91 g, 24.04 mmol, 4 equiv) portionwise at 0 °C with resulting gas evolution. Once the effervescence vanished, the resulting mixture was transferred into a new MW vial which was sealed and heated under MW conditions for 15 min at 90 °C. The reaction mixture was quenched with water (25 mL) and volatiles were removed under reduced pressure. The aqueous layer was extracted with DCM (2 x 40 mL) then the combined organic layers were successively washed with a saturated aqueous solution of sodium bicarbonate and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude material was purified by column chromatography on silica gel using EtOAc–MeOH–TEA (40:10:1) as eluent to provide the titled compound **5c** as a pale yellow oil which slowly solidified (0.68 g, 52%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.58 (s, 1H), 6.55 (s, 1H), 5.88 (s, 2H), 3.34 (br t, *J* = 8.4, 1H), 3.14–3.18 (m, 1H), 3.06–3.10 (m, 1H), 2.97–3.04 (m, 1H), 2.72 (br dt, *J* = 16.3 and *J* = 3.8, 1H), 2.52 (q, *J* = 8.7, 1H), 2.25–2.32 (m, 1H), 1.88–1.97 (m, 1H), 1.81–1.86 (m, 1H), 1.65–1.73 (m, 1H). All the other analytical data were consistent with the ones already reported in the literature [2].

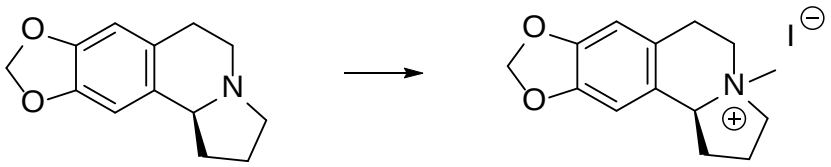
4-Methyl-8,9-methylenedioxy-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinolinium iodide 6c



5c **6c**

Starting from indolizidine derivative **5c** (123 mg, 0.57 mmol) and following general procedure 1, indolizidinium salt **6c** was obtained as a white solid (156 mg, 77%); mp 158–160 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.84 (s, 1H), 6.81 (s, 1H), 5.99 (s, 2H), 4.60 (br t, J = 9.1, 1H), 3.44–3.84 (m, 4H), 2.86–3.14 (m, 5H), 2.57–2.72 (m, 1H), 1.85–2.18 (m, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 147.8, 147.1, 123.9, 122.5, 109.0, 107.5, 102.0, 72.0, 66.3, 53.3, 48.2, 32.8, 24.2, 20.3; HRMS (APPI): M^+ found 232.1331. $C_{14}H_{18}NO_2$ requires 232.1335.

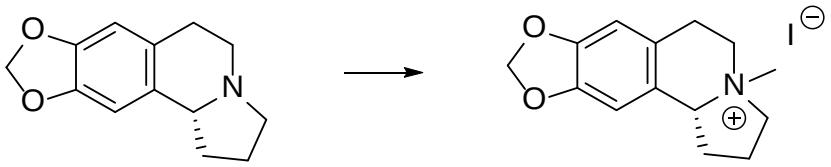
(*S*)-4-Methyl-8,9-methylenedioxy-2,3,4,5,6,10b-hexahydro-1*H*-pyrrolo[2,1-*a*]isoquinolin-4-ium iodide (*S*)-6c



(*S*)-5c **(*S*)-6c**

Starting from indolizidine derivative (*S*)-**5c** (96 mg, 0.44 mmol) and following general procedure 2, indolizidinium salt (*S*)-**6c** was obtained as a white solid (95 mg, 60%); ^{1}H NMR (600 MHz, DMSO-d_6): δ 6.86 (s, 1H), 6.83 (s, 1H), 6.01 (s, 2H), 4.61 (br t, J = 9.3, 1H), 3.69–3.82 (m, 2H), 3.60 (dt, J = 12.3 and J = 5.2, 1H), 3.47–3.53 (m, 1H), 3.05–3.14 (m, 4H), 2.91–2.98 (m, 1H), 2.61–2.69 (m, 1H), 2.08–2.16 (m, 2H), 1.89–1.98 (m, 1H). The other analytical data perfectly matched the ones reported above for the racemic compound **6c**.

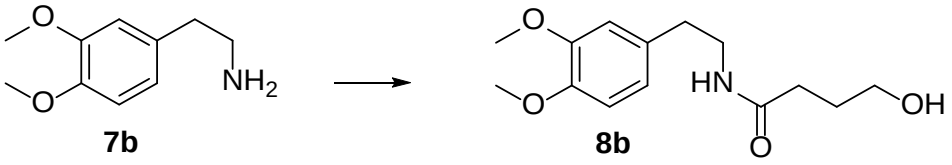
(*R*)-4-Methyl-8,9-methylenedioxy-2,3,4,5,6,10b-hexahydro-1*H*-pyrrolo[2,1-*a*]isoquinolin-4-ium iodide (*R*)-6c



(*R*)-5c **(*R*)-6c**

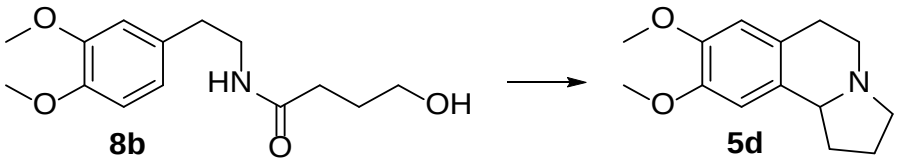
Starting from indolizidine derivative (*R*)-**5c** (96 mg, 0.44 mmol) and following general procedure 2, indolizidinium salt (*R*)-**6c** was obtained as a white solid (86 mg, 54%); ^{1}H NMR (600 MHz, DMSO-d_6): δ 6.86 (s, 1H), 6.83 (s, 1H), 6.01 (s, 2H), 4.60 (br t, J = 9.3, 1H), 3.69–3.82 (m, 2H), 3.60 (dt, J = 12.3 and J = 5.2, 1H), 3.48–3.54 (m, 1H), 3.06–3.14 (m, 4H), 2.91–2.98 (m, 1H), 2.62–2.70 (m, 1H), 2.09–2.17 (m, 2H), 1.90–1.98 (m, 1H). The other analytical data perfectly matched the ones reported above for the racemic compound **6c**.

***N*-(3,4-Dimethoxyphenethyl)-4-hydroxybutanamide 8b** [2]



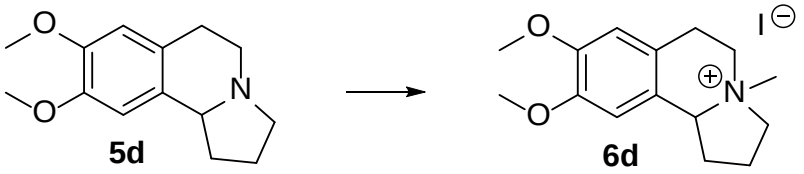
To a MW vial were successively added 2-(3,4-dimethoxyphenyl)ethylamine **7b** (2.9 g, 16.0 mmol, 1 equiv) and γ-butyrolactone (1.5 g, 17.6 mmol, 1.1 equiv) at room temperature. The MW vial was sealed and heated under MW conditions for 15 min at 150 °C. The resulting viscous orange oil was purified by column chromatography on silica gel using DCM–MeOH (10:1) as eluent to provide the titled compound **8b** as an off-white solid (3.0 g, 70%). All analytical data were consistent with the ones already reported in the literature [2].

8,9-Dimethoxy-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline 5d [2]



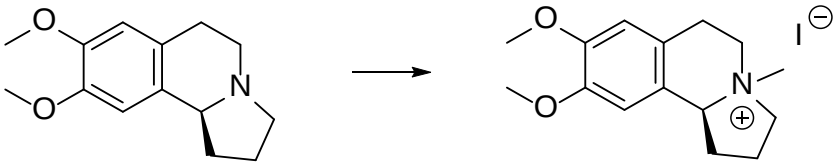
To a MW vial were successively added compound **8b** (2.5 g, 9.4 mmol, 1 equiv), acetonitrile (9.6 mL) and phosphorous (III) oxychloride (4.4 mL, 46.8 mmol, 5 equiv) at room temperature. The MW vial was sealed and heated under MW conditions for 15 min at 150 °C. Volatiles were removed under reduced pressure and the resulting material was dissolved in an AcOH–MeOH (1:12, 13 mL) mixture prior to addition of sodium borohydride (1.4 g, 37.4 mmol, 4 equiv) portionwise at 0 °C with resulting gas evolution. Once the effervescence vanished, the resulting mixture was transferred into a new MW vial which was sealed and heated under MW conditions for 15 min at 90 °C. The reaction mixture was quenched with water (30 mL) and volatiles were removed under reduced pressure. The aqueous layer was extracted with DCM (2 x 50 mL) then the combined organic layers were successively washed with a saturated aqueous solution of sodium bicarbonate and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude material was purified by column chromatography on silica gel using EtOAc–MeOH–TEA (40:10:1) as eluent to provide the titled compound **5d** as a white solid (0.64 g, 30%). All analytical data were consistent with the ones already reported in the literature [2].

8,9-Dimethoxy-4-methyl-2,3,4,5,6,10b-hexahydro-1*H*-pyrrolo[2,1-*a*]isoquinolin-4-ium iodide 6d [8]



Starting from indolizidine derivative **5d** (104 mg, 0.45 mmol) and following general procedure 1, indolizidinium salt **6d** was obtained as a white solid (117 mg, 70%); dec 210 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.66 (s, 1H), 6.63 (s, 1H), 5.12 (br t, *J* = 9.4, 1H), 4.30–4.45 (m, 1H), 4.18–4.29 (m, 1H), 3.97–4.08 (m, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.50–3.64 (m, 1H), 3.47 (s, 3H), 3.19–3.33 (m, 1H), 2.98–3.11 (m, 1H), 2.80–2.95 (m, 1H), 2.41–2.67 (m, 1H), 2.18–2.34 (m, 1H), 1.93–2.09 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 149.5, 149.1, 121.5, 119.0, 111.3, 109.9, 72.6, 66.8, 56.6, 56.4, 53.9, 48.1, 32.8, 24.1, 20.4; HRMS (APPI): M^+ found 248.1648. $C_{15}H_{22}NO_2$ requires 248.1649.

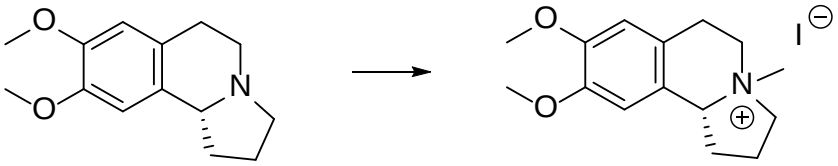
(*S*)-8,9-Dimethoxy-4-methyl-2,3,4,5,6,10b-hexahydro-1*H*-pyrrolo[2,1-*a*]isoquinolin-4-ium iodide (*S*)-6d



(*S*)-5d **(*S*)-6d**

Starting from indolizidine derivative (*S*)-**5d** (69 mg, 0.30 mmol) and following general procedure 2, indolizidinium salt (*S*)-**6d** was obtained as a white solid (47 mg, 42%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.67 (s, 1H), 6.63 (s, 1H), 5.13 (br t, *J* = 9.4, 1H), 4.33–4.44 (m, 1H), 4.20–4.29 (m, 1H), 3.98–4.07 (m, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.51–3.62 (m, 1H), 3.48 (s, 3H), 3.18–3.32 (m, 1H), 2.98–3.09 (m, 1H), 2.80–2.93 (m, 1H), 2.42–2.54 (m, 1H), 2.17–2.30 (m, 1H), 1.93–2.07 (m, 1H). The other analytical data perfectly matched the ones reported above for the racemic compound **6d**.

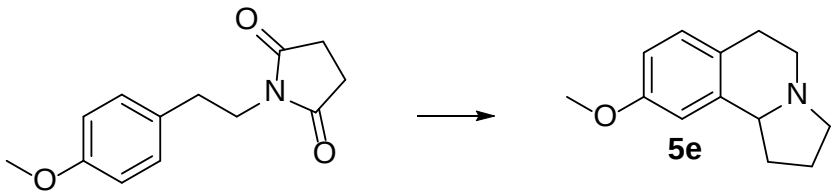
(*R*)-8,9-Dimethoxy-4-methyl-2,3,4,5,6,10b-hexahydro-1*H*-pyrrolo[2,1-*a*]isoquinolin-4-ium iodide (*R*)-6d



(*R*)-5d **(*R*)-6d**

Starting from indolizidine derivative (*R*)-**5d** (70 mg, 0.30 mmol) and following general procedure 2, indolizidinium salt (*R*)-**6d** was obtained as a white solid (50 mg, 44%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.67 (s, 1H), 6.62 (s, 1H), 5.14 (br t, *J* = 9.4, 1H), 4.40–4.51 (m, 1H), 4.20–4.29 (m, 1H), 3.95–4.03 (m, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.48–3.61 (m, 4H), 3.20–3.32 (m, 1H), 2.99–3.08 (m, 1H), 2.81–2.93 (m, 1H), 2.45–2.59 (m, 1H), 2.18–2.32 (m, 1H), 1.95–2.09 (m, 1H). The other analytical data perfectly matched the ones reported above for the racemic compound **6d**.

9-Methoxy-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline 5e [3]



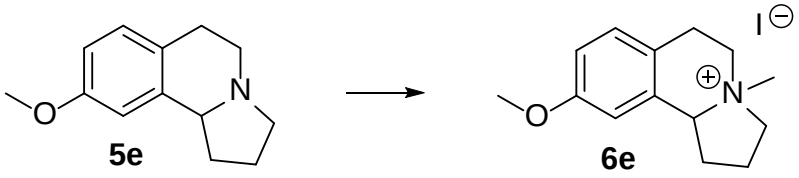
To a cooled solution of 1-(4-methoxyphenethyl)pyrrolidine-2,5-dione [9] (10.8 g, 46.3 mmol, 1 equiv) in EtOH (200 mL), saturated aqueous sodium bicarbonate (30 mL) and water (20 mL) at 0 °C was added sodium borohydride (8.76 g, 231.5 mmol, 5 equiv) in small portions. The reaction mixture was stirred at this temperature for 1 h then volatiles were removed under reduced pressure. After addition of water (300 mL), the aqueous layer was extracted with EtOAc (3 x 150 mL) then the combined organic layers were washed with brine (2 x 50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure.

To a solution of the resulting crude product in dry DCM (300 mL) was slowly added TfOH (40.9 mL, 463.0 mmol, 10 equiv) at room temperature. The mixture was heated under reflux conditions for 1 h. Upon cooling, the mixture was neutralized with a saturated aqueous sodium bicarbonate solution (500 mL) and volatiles were removed under reduced pressure. The resulting aqueous layer was extracted with EtOAc (3 x 150 mL) then the combined organic layers were washed with brine (2 x 50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford an off-white solid.

To a cooled solution of the resulting crude in dry THF (200 mL) at 0 °C was slowly added LAH (3.51 g, 92.6 mmol, 2 equiv) in small portions. The reaction mixture was allowed to warm up to room temperature, stirred at this temperature for 1 h then was cooled to 0 °C. Et_2O (200 mL), water (3.44 mL), 15% aqueous potassium hydroxide (3.44 mL) and water (7 mL) were successively added under vigorous stirring. The mixture was dried over $MgSO_4$, filtered and concentrated onto Celite® and purified by column chromatography on silica gel using EtOAc–TEA (25:1) as eluent to provide the titled compound **5e** as a colorless oil which solidified when stored in the refrigerator (6.98 g, 76% over 3 steps); R_f = 0.36 (EtOAc–TEA, 25:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.02 (d, J = 8.4, 1H), 6.70 (dd, J = 8.4 and J = 2.7, 1H), 6.62 (d, J = 2.9, 1H), 3.78 (s, 3H), 3.38–3.43 (m, 1H), 3.16–3.21 (m, 1H), 2.99–3.11 (m, 2H), 2.73–2.78 (m, 1H), 2.59–2.64 (m, 1H), 2.49–2.55 (m, 1H), 2.30–2.36 (m, 1H), 1.82–1.98 (m, 2H), 1.70–1.78 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.8, 140.2, 129.5, 126.5, 111.9, 111.2, 63.7, 55.4, 53.5, 48.8, 30.4, 27.9, 22.3; LRMS m/z (ESI^+): 204 $[M+H]^+$.[2]

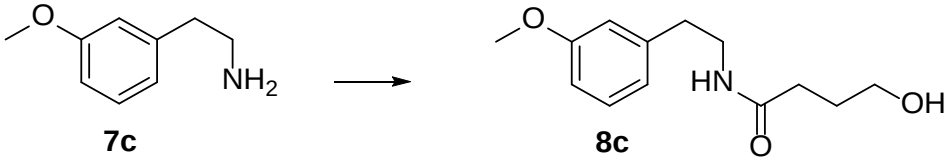
9-Methoxy-4-methyl-2,3,4,5,6,10b-hexahydro-1*H*-pyrrolo[2,1-*a*]isoquinolin-4-ium iodide 6e

[2] To a solution of **5e** (915 mg, 4.5 mmol, 1 equiv) in 1,4-dioxane (6.5 mL) was slowly added hydrochloric acid (4 M in 1,4-dioxane, 5.4 mmol, 1.35 mL, 1.2 equiv) at room temperature under nitrogen atmosphere. The precipitate was filtered and washed with cold 1,4-dioxane to provide **5e**·**HCl** (845 mg, 78%) as a white solid. For the binding and functional assays, **5e**·**HCl** was tested instead of **5e**.



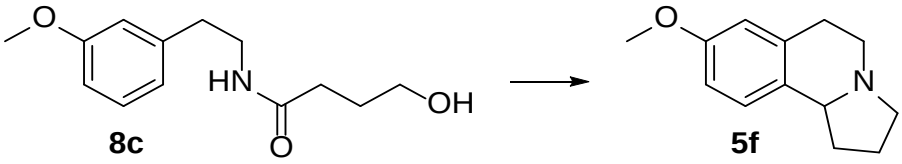
Starting from indolizidine derivative **5e** (285 mg, 1.40 mmol) and following general procedure 1, indolizidinium salt **6e** was obtained as a white solid (393 mg, 81%); mp 153–155 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.14 (d, *J* = 8.4, 1H), 6.86 (dd, *J* = 8.4 and *J* = 2.7, 1H), 6.68 (d, *J* = 2.9, 1H), 5.18–5.23 (m, 1H), 4.36–4.44 (m, 1H), 4.23–4.29 (m, 1H), 4.02–4.08 (m, 1H), 3.78 (s, 3H), 3.55–3.62 (m, 1H), 3.50 (s, 3H), 3.20–3.30 (m, 1H), 3.02–3.10 (m, 1H), 2.84–2.92 (m, 1H), 2.46–2.54 (m, 1H), 2.21–2.30 (m, 1H), 1.99–2.08 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 159.1, 130.5, 130.4, 118.4, 115.9, 111.7, 72.7, 66.7, 55.6, 53.9, 47.9, 32.5, 23.3, 20.1; HRMS (APPI): M^+ found 218.1540. $C_{14}H_{20}NO$ requires 218.1539.

***N*-(3-Methoxyphenethyl)-4-hydroxybutanamide 8c**



To a MW vial were successively added 2-(3-methoxyphenyl)ethylamine **7c** (1.01 g, 6.68 mmol, 1 equiv) and γ-butyrolactone (0.63 g, 7.35 mmol, 1.1 equiv) at room temperature. The MW vial was sealed and heated under MW conditions for 15 min at 150 °C. The resulting viscous yellow oil was purified by column chromatography on silica gel using a gradient elution (DCM–MeOH, 300:15 to 100:10) to furnish the titled compound **8c** as a colorless oil (1.34 g, 85%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.23 (t, *J* = 7.9, 1H), 6.77–6.80 (m, 2H), 6.75 (t, *J* = 2.1, 1H), 5.93 (br s, 1H), 3.81 (s, 3H), 3.67 (t, *J* = 5.7, 2H), 3.53 (q, *J* = 6.6, 2H), 2.93 (br s, 1H), 2.82 (t, *J* = 6.9, 2H), 2.33 (dd, *J* = 7.4 and *J* = 6.2, 2H), 1.83–1.88 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 173.7, 160.0, 140.5, 129.8, 121.2, 114.7, 112.0, 62.4, 55.3, 40.8, 35.7, 34.1, 28.1.

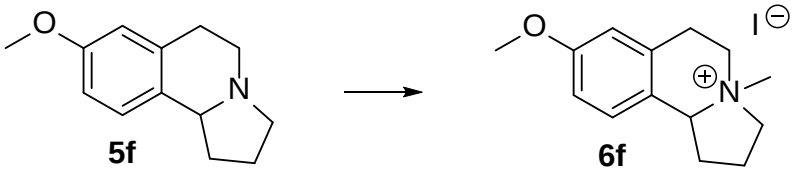
8-Methoxy-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline 5f [10,11]



To a MW vial were successively added compound **8c** (1.33 g, 5.60 mmol, 1 equiv), acetonitrile (11 mL) and phosphorous (III) oxychloride (2.62 mL, 28.02 mmol, 5 equiv) at room temperature. The MW vial was sealed and heated under MW conditions for 45 min at 160 °C. Volatiles were removed under reduced pressure and the resulting material was dissolved in MeOH (15 mL) prior to addition of sodium borohydride (0.85 g, 22.42 mmol, 4 equiv) portionwise at 0 °C. The reaction mixture was stirred at this temperature for 0.5 h then allowed to warm up to room temperature and stirred at this temperature for 3.5 h prior to quench with water (25 mL). Volatiles were removed

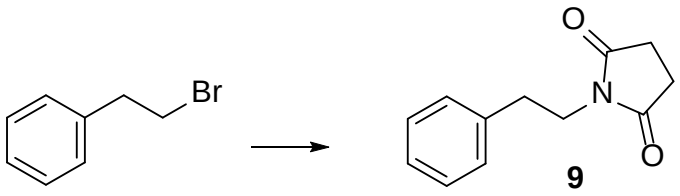
under reduced pressure then the aqueous layer was extracted with DCM (3 x 40 mL) and the combined organic layers were successively washed with a saturated aqueous solution of sodium bicarbonate and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude material was purified by column chromatography on silica gel using a gradient elution (EtOAc–MeOH–TEA, 25:1:1 to 25:2:1) to provide the titled compound **5f** as a colorless oil (0.71 g, 62%); R_f = 0.35 (EtOAc–MeOH–TEA, 25:2:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.99 (d, *J* = 8.4, 1H), 6.71 (dd, *J* = 8.4 and *J* = 2.7, 1H), 6.66 (d, *J* = 2.7, 1H), 3.78 (s, 3H), 3.39–3.45 (m, 1H), 3.16–3.20 (m, 1H), 3.04–3.11 (m, 2H), 2.80–2.86 (m, 1H), 2.64–2.70 (m, 1H), 2.58 (q, *J* = 8.7, 1H), 2.31–2.38 (m, 1H), 1.83–1.99 (m, 2H), 1.68–1.75 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 158.0, 135.6, 131.2 (br s), 126.7, 113.4, 112.1, 63.1, 55.4, 53.5, 48.6, 30.6, 29.0, 22.4.

8-Methoxy-4-methyl-2,3,4,5,6,10b-hexahydro-1*H*-pyrrolo[2,1-*a*]isoquinolin-4-ium iodide 6f [10]



Starting from indolizidine derivative **5f** (95 mg, 0.47 mmol) and following general procedure 1, indolizidinium salt **6f** was obtained as a white solid (110 mg, 69%); mp 185–187 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.10 (d, *J* = 8.5, 1H), 6.83 (dd, *J* = 8.6 and *J* = 2.6, 1H), 6.73 (d, *J* = 2.5, 1H), 5.12 (t, *J* = 9.5, 1H), 4.21–4.37 (m, 2H), 4.04–4.12 (m, 1H), 3.78 (s, 3H), 3.61 (dt, *J* = 12.5 and *J* = 5.4, 1H), 3.47 (s, 3H), 3.24–3.36 (m, 1H), 3.06–3.16 (m, 1H), 2.74–2.86 (m, 1H), 2.39–2.53 (m, 1H), 2.18–2.32 (m, 1H), 1.93–2.06 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 159.8, 129.0, 128.5, 121.4, 114.8, 113.5, 72.6, 66.7, 55.6, 53.6, 48.1, 32.8, 24.4, 20.2; HRMS (APPI): M^+ found 218.1537. $C_{14}H_{20}NO$ requires 218.1539.

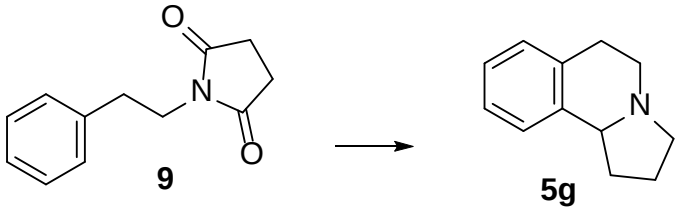
1-Phenethylpyrrolidine-2,5-dione 9



To a solution of succinimide (1.02 g, 10.29 mmol, 1 equiv) in dry DMF (18 mL) was added sodium hydride (453 mg, 60% dispersion in oil, 11.32 mmol, 1.1 equiv) at room temperature. The reaction mixture was stirred for 1 h at this temperature then a solution of 2-phenylethylbromide (2.10 g, 11.32 mmol, 1.1 equiv) in dry DMF (6 mL) was added dropwise. The reaction mixture was heated 50 °C for 2 h then stirred at room temperature for 16 h. After addition of EtOAc, the organic layer was washed four times with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting white crude solid was purified by column chromatography on silica gel

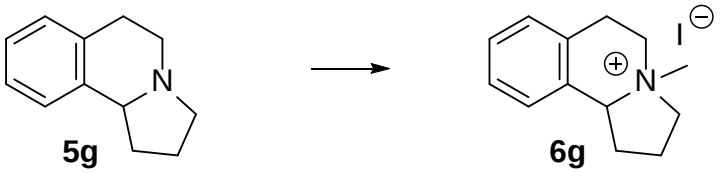
using a gradient elution (EtOAc–PE, 4:3 to 2:1) to afford pure phenethylamide **9** (1.52 g, 73%) as a white solid; R_f = 0.45 (EtOAc–PE, 4:3); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.17–7.32 (m, 5H), 3.69–3.78 (m, 2H), 2.82–2.91 (m, 2H), 2.64 (s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.1 (2C), 137.9, 129.0 (2C), 128.7 (2C), 126.8, 40.1, 33.7, 28.3 (2C).

1,2,3,5,6,10b-Hexahydropyrrolo[2,1-*a*]isoquinoline 5g [12]



TfOH (708 μL, 8 mmol, 8 equiv) was added to phenethylamide **9** (204 mg, 1 mmol, 1 equiv) at room temperature. The resulting mixture was heated under MW conditions for 80 min at 110 °C then cooled to room temperature and diluted with dry DCM (12 mL) prior to add sodium borohydride (152 mg, 4 mmol, 4 equiv) followed by MeOH (6 mL). The reaction mixture was stirred for 2 h at room temperature then acetone was added prior to remove volatiles under reduced pressure. The resulting crude was taken up in EtOAc then washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to provide an off-white solid which was dissolved in dry THF (15 mL) and cooled to 0 °C prior to add LAH (95 mg, 2.5 mmol, 2.5 equiv) portionwise. The mixture was allowed to warm up to 50 °C and stirred at this temperature for 2 h then cooled to 0 °C. An aqueous solution of Rochelle salt (2 M) was carefully added and the reaction mixture was allowed to stir at room temperature for 1 h. The resulting solution was made alkaline by addition of an aqueous solution of NaOH (3 M) and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by column chromatography on silica gel using EtOAc–TEA (25:1) as eluent to lead to pure indolizidine **5g** (116 mg, 67% over two steps) as a colorless oil; R_f = 0.30 (EtOAc–TEA, 25:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.04–7.18 (m, 4H), 3.39–3.44 (m, 1H), 3.18–3.23 (m, 1H), 3.07–3.15 (m, 2H), 2.80–2.86 (m, 1H), 2.64 (ddd, J = 11.2 and J = 10.5 and J = 4.8, 1H), 2.52 (q, J = 10.2, 1H), 2.32–2.40 (m, 1H), 1.83–1.99 (m, 2H), 1.70–1.78 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 139.2, 134.4, 128.6, 126.1, 125.9, 125.7, 63.6, 53.6, 48.7, 30.3, 28.9, 22.3.

4-Methyl-2,3,4,5,6,10b-hexahydro-1*H*-pyrrolo[2,1-*a*]isoquinolin-4-ium iodide 6g

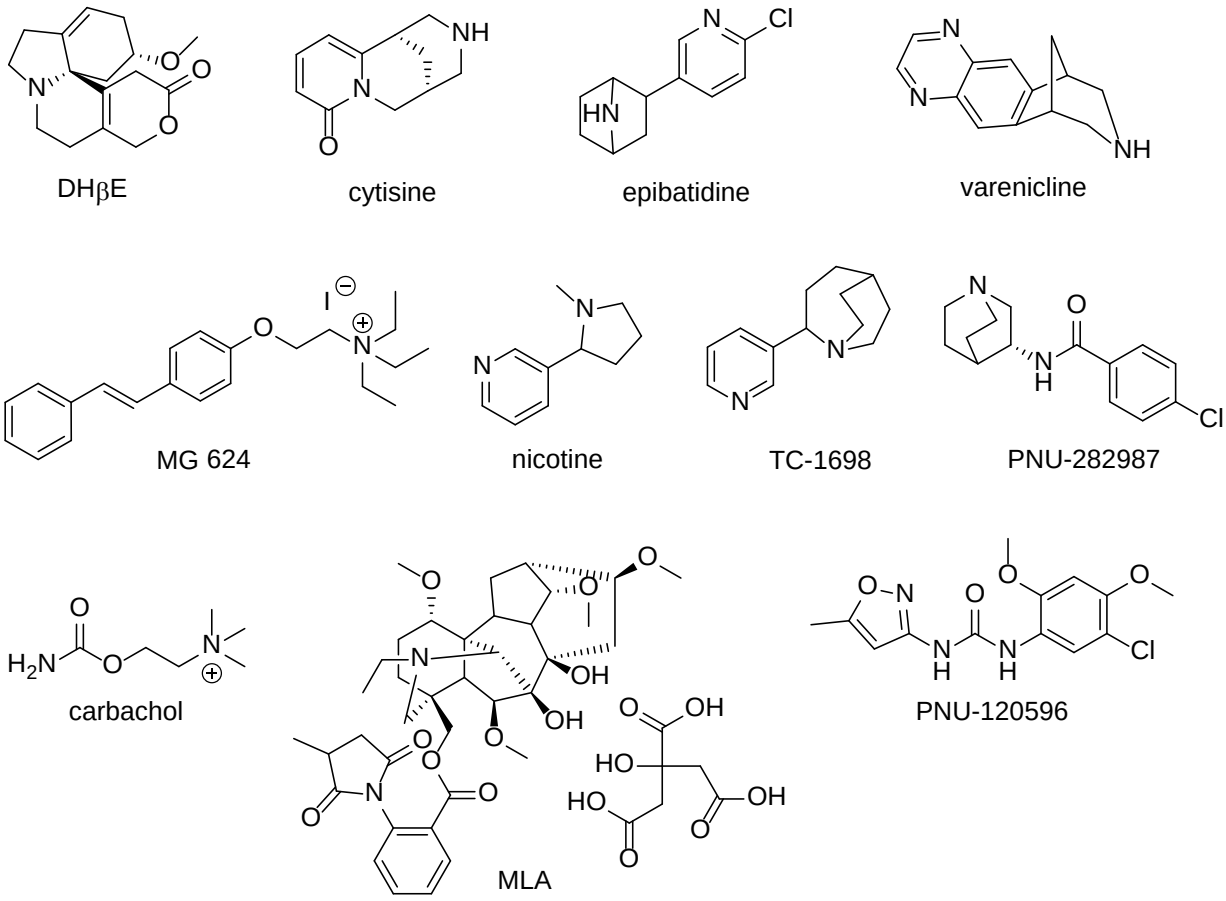


Starting from indolizidine derivative **5g** (100 mg, 0.58 mmol) and following general procedure 1, indolizidinium salt **6g** was obtained as a white solid (143 mg, 79%); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.22–7.32 (m, 3H), 7.19 (dd, *J* = 7.4 and *J* = 1.6, 1H), 5.24 (dd, *J* = 10.7 and *J* = 8.5, 1H), 4.34–4.41 (m, 1H), 4.25–4.31 (m, 1H), 4.09–4.13 (m, 1H), 3.63 (dt, *J* = 12.7 and *J* = 5.4, 1H), 3.50 (s, 3H), 3.28–3.37 (m, 1H), 3.10–3.18 (m, 1H), 2.81–2.90 (m, 1H), 2.44–2.54 (m, 1H), 2.22–2.31 (m, 1H), 1.99–2.09 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 129.5, 129.3, 128.9, 128.1, 127.0, 72.7, 66.8, 53.8, 48.1, 32.7, 24.1, 20.2; HRMS (APPI): M^+ found 188.1430. $C_{13}H_{18}N$ requires 188. 1434.

3 Materials, methods and abbreviations (in vitro pharmacology)

Abbreviations. DHβE: dihydro-β-erythroidine; nAChR: nicotinic acetylcholine receptor; MLA: methyllycaconitine; HEK: human embryonic kidney; S.E.M.: standard error of the mean; K_i: binding affinity value, ACh: acetylcholine; FMP: FLIPR Membrane Potential Blue; cDNA: complementary DNA; DMEM: Dulbecco's modified eagle's medium; FBS: fetal bovine serum.

Materials.



Culture media, serum, antibiotics and buffers for cell culture were obtained from Invitrogen (Paisley, UK). Polyfect transfection reagent, Fluo–4/AM dye and FLIPR Membrane Potential Blue

assay dye were purchased from Qiagen (Hilden, Germany), Molecular Probes (Eugene, OR) and Molecular Devices (Crawley, UK), respectively. ACh, (*S*)-nicotine and carbachol were purchased from Sigma (St. Louis, MO), (±)-epibatidine, (–)-cytisine, varenicline, TC-1698, PNU-282987, PNU-120596, MLA, α-bungarotoxin and MG 624 were purchased from Tocris Cookson (Bristol, UK), and [^{3}H]epibatidine was obtained from PerkinElmer (Waltham, MA). The HEK293 cell lines stably expressing the rat α3β4, α4β4 and α4β2 nAChRs were generous gifts from Drs. K. J. Kellar and Y. Xiao (Georgetown University School of Medicine, Washington, DC) and Dr. J. H. Steinbach (Washington University School of Medicine, St. Louis, MO), and the HEK293T cell line stably expressing the mouse α4β2 nAChR was a generous gift from Dr. J. A. Stitzel (University of Colorado, Boulder, CO) [13–16]. The cDNAs for human α7 and human full-length Ric-3 were generously provided by Dr. J. Lindstrom (University of Pennsylvania Medical School, Philadelphia, PA) and Dr. N. S. Millar (University College London, London, UK), respectively, and the cDNA for human NACHO was purchased from Origene (#SC112910, Rockville, MD).

Methods.

Molecular Biology. The construction of the hα7-pCIneo plasmid has been described previously [17]. The cDNA for human NACHO [18] was subcloned into the CMV multiple cloning site of the bistronic mammalian expression vector pBudCE4.1 (Sigma, St. Louis, MO) by use of the restriction enzymes *Hind*III and *Xba*I, after which the cDNA for full-length Ric-3 [19] was subcloned into the EF-1α promoter-associated multi-cloning site of the vector by use of the restriction enzymes *Not*I and *Xho*I, thus yielding the Ric-3/NACHO-pBudCE4.1 construct. The integrity of and the absence of unwanted mutations in this construct was verified by DNA sequencing (Eurofins MWG Operon, Martinsried, Germany).

Cell culture. All cell lines were cultured at 37 ºC in a humidified 5% CO_2 atmosphere. The HEK293 and tsA201 cells were maintained in culture medium [Dulbecco's Modified Eagle Medium Glutamax™-I (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin], rα3β4-, rα4β4- and rα4β2-HEK293 cells in culture medium supplemented with 1 mg/ml G-418, and the mα4β2-HEK293T cells in culture medium supplemented with 100 μg/ml zeocin and 500 μg/ml hygromycin B. The stable hα7$^{\text{Ric-3/NACHO}}$-HEK293 cell line was maintained in DMEM supplemented with 5% dialyzed FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml zeocin and 1 mg/ml G-418.

Construction of a monoclonal stable hα7$^{\text{Ric-3/NACHO}}$ -HEK293 cell line. HEK293 cells were co-transfected with hα7-pCIneo and Ric-3/NACHO-pBudCE4.1 plasmids using the PolyFect transfection reagent and cultured in DMEM supplemented with 5% dialyzed FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml zeocin and 1 mg/ml G-418. After approximately three weeks in culture, antibiotic-resistant colonies were picked and cultured further under antibiotic selection. The various clones were screened for functionality in the Ca^{2+}/Fluo-4 assay and one of the functional clones was selected and subsequently used for the functional characterization

of a series of reference nAChR ligands and selected test compounds in the Ca^{2+}/Fluo-4 assay (see below for more details).

The [^{3}H]epibatidine binding assay. The binding properties of the compounds were determined at membranes from the stable rα3β4-, rα4β4- and rα4β2-HEK293 cell lines in a [^{3}H]epibatidine binding assay essentially as previously described [20]. Briefly, cells were harvested at 80-90% confluency and scraped into ice-cold assay buffer (140 mM NaCl, 1.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM HEPES, pH 7.4), homogenized using a Polytron for 10 s, and centrifuged for 20 min at 50000g at 4 ºC. The pellets were re-suspended in fresh ice-cold assay buffer, homogenized, and centrifuged at 50000g for another 20 min at 4 ºC, after which the pellets were stored at -80 °C. On the day of the assay, pellets were re-suspended in the assay buffer. In saturation binding experiments the membranes were incubated with various concentrations of [^{3}H]epibatidine (0.1 pM – 5 nM) in the absence (total binding) or presence of 100 μM (*S*)-nicotine (non-specific binding) in assay volumes ranging from 2 to 5 mL. In competition binding experiments, membranes were incubated with a fixed [^{3}H]epibatidine concentration (α4β2: 25 pM, α3β4 and α4β4: 100 pM) and various concentrations of the test compounds in assay volumes of 2 mL. The reactions were incubated for 4 h at room temperature while gently shaking. Whatman GF/C filters (Sigma) were presoaked for 1 h in a 0.2% polyethyleneimine solution, and binding was terminated by filtration through these filters using a 48-well cell harvester and washing with 3 x 5 mL of ice-cold wash buffer (0.9% NaCl, 10 mM Tris-HCl, pH 7.4). Following this, the filters were dried and added to 3 mL of Opti-Fluor (PerkinElmer) in scintillation vials after which radioactivity was counted in a TriCarb4910 scintillation counter (PerkinElmer). The fraction of specifically bound [^{3}H]epibatidine was always <10% of the total amount of the radioligand in the assay.

The FLIPR Membrane Potential Blue (FMP) assay. The functional properties of the compounds were determined at the mα4β2-HEK293T- and rα3β4-HEK293-cell lines in the FMP assay essentially as previously described [20]. Briefly, the cells were split into poly-D-lysine-coated black 96-well plates (BD Biosciences, Palo Alto, CA) with clear bottom (6 x 10^4 cells/well). The following day, the culture medium was aspirated, and the cells were washed once with 100 μl assay buffer after which the cells were incubated in 100 μl assay buffer (140 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 11 mM HEPES, 10 mM D-Glucose, pH 7.4) supplemented with FMP dye (0.5 mg/ml) at 37 °C for 30 min. In the antagonist experiments, the antagonist was added to the assay buffer at this point. The 96-well plate was assayed in a FLEXStation^3 Benchtop Multi-Mode Microplate Reader (Molecular Devices) measuring emission [in fluorescence units (FU)] at 565 nm caused by excitation at 525 nm before and up to 90 s after addition of 33.3 μL assay buffer supplemented with test compound (in the agonist experiments) or with (*S*)-nicotine EC_{80} (in the antagonist experiments).

The Ca^{2+}/Fluo-4 assay. The functional characterization of various reference nAChR ligands and selected analogues at the human α7 nAChR was performed at the stable hα7$^{Ric\text{-}3/NACHO}$-HEK293 cell line in the Ca^{2+}/Fluo-4 assay. The cells were split into poly-D-lysine-coated black 96-well plates with clear bottom (6 x 10^4 cells/well). The following day, the culture medium was aspirated

and the cells were incubated in 50 µl loading buffer [Hanks' Buffered Salt Solution (HBSS) containing 20 mM HEPES, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 2.5 mM probenecid, pH 7.4] supplemented with 6 mM Fluo–4/AM supplemented at 37 °C for 1 h. Then the buffer was aspirated, the cells were washed once with 100 µl loading buffer, and then 100 µl assay buffer (loading buffer supplemented with 3 µM PNU-120596) was added to the cells. In the antagonist experiments, the antagonist was added to the assay buffer at this point. The 96-well plate was assayed in a FLEXStation^3 Benchtop Multi-Mode Microplate Reader measuring emission [in fluorescence units (FU)] at 525 nm caused by excitation at 485 nm before and up to 90 s after addition of 33.3 µl assay buffer supplemented with test compound (in the agonist experiments) or with ACh EC_{80} (in the antagonist experiments).

Data Analysis. Data from saturation binding experiments with [^{3}H]epibatidine at α3β4, α4β4 and α4β2 nAChRs were fitted to a single-site ligand binding model, and the dissociation constants (K_D) for the radioligand were determined by nonlinear regression. Data from the competition binding experiments were fitted to the equation %Bound = 100%Bound/[1+ $([L]/IC_{50})^{nH}$]. K_i values were calculated using the Cheng-Prusoff equation $K_i = IC_{50}/(1 + [RL]/K_D)$, where [RL] is the radioligand concentration, nH the Hill slope, and K_D the dissociation constant for the radioligand. The data from the functional experiments were fitted to sigmoidal curves with variable slopes using nonlinear regression, and EC_{50} values for agonists and IC_{50} values for antagonists were derived from these equations. The maximal responses (R_{max}) for agonists were derived from the fitted concentration-response curves, and these values were normalized to the R_{max} value obtained for the reference agonist at the same 96-well plate. Curves were generated by nonweighted least-squares fits using the program KaleidaGraph 3.08 (Synergy Software, Reading, PA).

Results. Binding properties the compounds at membranes from HEK293 cell lines stably expressing rat α4β2, α4β4 and α3β4 nAChRs in the [^{3}H]epibatidine competition binding assay. K_i values are given in µM with pK_i± S.E.M. values in brackets. Functional properties of the compounds at the stable mouse α4β2-HEK293T and rat α3β4-HEK293 cell lines in the FLIPR Membrane Potential Blue assay and at the stable hα7$^{Ric\text{-}3/NACHO}$-HEK293 cell line in the Ca^{2+}/Fluo-4 assay. EC_{50} and IC_{50} values are given in µM with pEC_{50} ± S.E.M. and pIC_{50} ± S.E.M. values in brackets, and R_{max} ± S.E.M. values for the agonists at the hα7 receptor are given in % of the ACh R_{max} obtained at the same plate. In the antagonist experiments, EC_{80} (EC_{70} – EC_{90}) concentration of (*S*)-nicotine (mα4β2 and rα3β4) and ACh (hα7) were used as agonists. The testing of the compounds at the stable hα7$^{Ric\text{-}3/NACHO}$-HEK293 cell line in the Ca^{2+}/Fluo-4 assay was performed in the presence of 3 µM PNU-120596. The data were the means of 3-5 individual experiments performed in duplicate.

4 Binding characterization (K_i) at the α4β2, α4β4 and α3β4 nAChRs

Compounds	α4β2	α4β4	α3β4
(*S*)-Nicotine	0.0042 [8.37 ± 0.06]	0.44 [7.36 ± 0.04]	0.21 [6.68 ± 0.03]
DHβE	0.65 [6.18 ± 0.08]	~25 [~4.6][a]	~100 [~4.0][a]
1	0.87 [6.06 ± 0.06]	~300 [~3.5]	~300 [~3.5]
2a	0.63 [6.20 ± 0.09]	~50 [~4.3]	~100 [~4.0]
2b	9.7 [5.01 ± 0.07]	>300 [<3.5]	>300 [<3.5]
2c	14 [4.85 ± 0.11]	~300 [~3.5]	~300 [~3.5]
2d	~30 [~4.5]	~300 [~3.5]	>300 [<3.5]
2e	~30 [~4.5]	>300 [<3.5]	~300 [~3.5]
3a	2.9 [5.54 ± 0.07]	~300 [~3.5]	~300 [~3.5]
3b	15 [4.81 ± 0.09]	~300 [~3.5]	~300 [~3.5]
3c	1.7 [5.77 ± 0.06]	~50 [~4.3]	~300 [~3.5]
3d	~100 [~4.0][a]	>100 [<4.0]	>100 [<4.0]
3e	1.7 [5.76 ± 0.08]	~100 [~4.0]	~300 [~3.5]
3f	2.6 [5.59 ± 0.04]	~25 [~4.6][a]	~100 [~4.0][a]
4a	0.47 [6.33 ± 0.11]	~50 [~4.3]	~30 [~4.5]
4b	4.5 [5.35 ± 0.13]	17 [4.79 ± 0.08]	~30 [~4.5]
4c	0.38 [6.42 ± 0.15]	~30 [~4.5]	~50 [~4.3]
4d	>100 [<4.0]	>100 [<4.0]	>100 [<4.0]
4e	0.40 [6.39 ± 0.07]	8.9 [5.05 ± 0.06]	~25 [~4.6][a]
4f	0.68 [6.17 ± 0.06]	1.5 [5.83 ± 0.07]	9.3 [5.03 ± 0.16]
5a	6.3 [5.20 ± 0.06]	~300 [~3.5][a]	~100 [~4.0][a]
5b	~50 [~4.3]	~300 [~3.5]	~300 [~3.5]
5c	0.50 [6.30 ± 0.08]	9.3 [5.03 ± 0.05]	>100 [<4.0]
(*R*)-**5c**	0.17 [6.78 ± 0.06]	6.6 [5.18 ± 0.04]	~100 [~4.0][a]
(*S*)-**5c**	4.5 [5.35 ± 0.07]	~100 [~4.0][a]	>100 [<4.0]
5d	4.6 [5.26 ± 0.09]	~100 [~4.0][a]	~100 [~4.0][a]
(*R*)-**5d**	2.5 [5.60 ± 0.03]	~100 [~4.0][a]	~100 [~4.0][a]
(*S*)-**5d**	>100 [<4.0]	>100 [<4.0]	>100 [<4.0]
5e	1.4 [5.86 ± 0.08]	3.3 [5.49 ± 0.01]	~100 [~4.0]
5f	~25 [~4.6][a]	~100 [~4.0][a]	>100 [<4.0]
5g	8.5 [5.07 ± 0.05]	~100 [~4.0][a]	~100 [~4.0][a]

6a	2.1 [5.65 ± 0.04]	~300 [~3.5]	~300 [~3.5]
6b	11 [4.96 ± 0.07]	~300 [~3.5]	~300 [~3.5]
6c	0.14 [6.87 ± 0.09]	~50 [~4.3][a]	~30 [~4.5][a]
(*R*)-**6c**	0.045 [7.34 ± 0.09]	2.7 [5.17 ± 0.04]	11 [4.94 ± 0.07]
(*S*)-**6c**	~25 [~4.6][a]	~25 [~4.6]	~25 [~4.6]
6d	2.4 [5.62 ± 0.05]	~30 [~4.5][a]	~50 [~4.3][a]
(*R*)-**6d**	2.4 [5.61 ± 0.07]	~25 [~4.6][a]	~25 [~4.6][a]
(*S*)-**6d**	~25 [~4.6][a]	7.5 [5.12 ± 0.05]	~25 [~4.6][a]
6e	0.23 [6.64 ± 0.06]	0.92 [6.04 ± 0.04]	9.3 [5.03 ± 0.07]
6f	3.6 [5.44 ± 0.02]	12 [4.90 ± 0.06]	~25 [~4.6][a]
6g	17 [4.76 ± 0.02]	~25 [~4.6][a]	~50 [~4.3][a]

[a] The concentration-inhibition curve was not complete within the concentration range tested. Thus, the K_i value is calculated from the IC_{50} value estimated from the fitted curve.

5 Functional characterization (EC_{50}, IC_{50}) at the α4β2 and α3β4 nAChRs

Compounds	α4β2		α3β4	
	EC_{50} [pEC_{50} ± S.E.M]	IC_{50} [pIC_{50} ± S.E.M]	EC_{50} [pEC_{50} ± S.E.M]	IC_{50} [pIC_{50} ± S.E.M]
(*S*)-Nicotine	0.35 [6.46 ± 0.09]		1.5 [5.82 ± 0.06]	
DHβE		0.60 [6.22 ± 0.08]		~100 [~4.0][a]
1		12 [4.92 ± 0.07]		~100 [~4.0][a]
2a		11 [4.96 ± 0.09]	>300 [<3.5][b]	>300 [<3.5][b]
2b		11 [4.97 ± 0.07]	>300 [<3.5][b]	>300 [<3.5][b]
2c		~30 [~4.5][a]	>300 [<3.5][b]	>300 [<3.5][b]
2d		~30 [~4.5][a]	>300 [<3.5][b]	>300 [<3.5][b]
2e		~30 [~4.5][a]	>300 [<3.5][b]	>300 [<3.5][b]
3a		24 [4.62 ± 0.08]		~100 [~4.0][a]
3b		23 [4.63 ± 0.09]		>300 [<3.5][a]
3c		2.6 [5.59 ± 0.11]		~300 [~3.5][a]
3d		>100 [< 4.0][b]	>100 [<4.0][b]	>100 [<4.0][b]
3e		4.4 [5.36 ± 0.11]		~100 [~4.0][a]
3f		6.2 [5.21 ± 0.06]	>100 [<4.0][b]	>100 [<4.0][b]
4a		7.2 [5.14 ± 0.09]	>300 [<3.5][b]	>300 [<3.5][b]
4b		~30 [~4.5][a]	>300 [<3.5][b]	>300 [<3.5][b]
4c		12 [4.92 ± 0.07]	>300 [<3.5][b]	>300 [<3.5][b]
4d	>100 [< 4.0][b]	>100 [< 4.0][b]	>100 [<4.0][b]	>100 [<4.0][b]
4e		1.2 [5.92 ± 0.03]	>100 [<4.0][b]	>100 [<4.0][b]
4f		1.4 [5.84 ± 0.04]		~100 [~ 4.0][a]
5a		~30 [~4.5][a]		~100 [~4.0][a]
5b		~50 [~4.3][a]	>300 [<3.5][b]	>300 [<3.5][b]
5c		2.3 [5.64 ± 0.07]		~100 [~4.0][a]
(*R*)-**5c**		1.3 [5.91 ± 0.06]	>100 [<4.0][b]	>100 [<4.0][b]
(*S*)-**5c**		~30 [~4.5][a]	>100 [<4.0][b]	>100 [<4.0][b]
5d		~30 [~4.5][a]	>100 [<4.0][b]	>100 [<4.0][b]
(*R*)-**5d**		16 [4.80 ± 0.03]	>100 [<4.0][b]	>100 [<4.0][b]
(*S*)-**5d**	>100 [< 4.0]	>100 [< 4.0][b]	>100 [<4.0][b]	>100 [<4.0][b]
5e		9.1 [5.04 ± 0.09]	>100 [<4.0][b]	>100 [<4.0][b]
5f		~100 [~4.0][a]	>100 [<4.0][b]	>100 [<4.0][b]

5g		15 [4.82 ± 0.07]	>100 [<4.0][b]	>100 [<4.0][b]
6a		3.1 [5.51 ± 0.09]		~300 [~3.5][a]
6b		5.7 [5.24 ± 0.07]		~300 [~3.5][a]
6c		0.52 [6.28 ± 0.05]		~30 [~4.5][a]
(*S*)-**6c**	>100 [< 4.0][b]	>100 [< 4.0][b]	>100 [<4.0][b]	>100 [<4.0][b]
(*R*)-**6c**		0.22 [6.66 ± 0.09]		~50 [~4.3][a]
6d		~30 [~4.5][a]		~100 [~4.0][a]
(*S*)-**6d**	>100 [< 4.0][b]	>100 [< 4.0][b]	>100 [<4.0][b]	>100 [<4.0][b]
(*R*)-**6d**		7.2 [5.15 ± 0.05]	>100 [<4.0][b]	>100 [<4.0][b]
6e		1.8 [5.74 ± 0.07]		~50 [~4.3][a]
6f		~30 [~4.5][a]		~100 [~4.0][a]
6g		~100 [~ 4.0][a]	>100 [<4.0][b]	>100 [<4.0][b]

[a] The concentration-inhibition curve was not complete within the concentration range tested. Thus, the IC_{50} value was estimated from the fitted curve.

[b] Inactive at this concentration both when tested as agonist and antagonist.

6 Functional characterization (EC_{50}, IC_{50}) at the α7 nAChR subtype

Compounds	EC_{50} [pEC_{50} ± S.E.M]	R_{max} ± S.E.M.	IC_{50} [pIC_{50} ± S.E.M]
(*S*)-Nicotine	0.82 [6.08 ± 0.08]	98 ± 3	
ACh	1.3 [5.89 ± 0.07]	100	
Carbachol	14 [4.85 ± 0.05]	77 ± 4	
(±)-Epibatidine	0.014 [7.86 ± 0.02]	97 ± 3	
(–)-Cytisine	0.95 [6.02 ± 0.03]	99 ± 4	
Varenicline	0.055 [7.26 ± 0.09]	95 ± 3	
TC-1698	0.021 [7.68 ± 0.06]	92 ± 3	
PNU-282987	0.024 [7.62 ± 0.06]	87 ± 4	
MLA			0.0026 [8.57 ± 0.06]
α-Bungarotoxin			0.0043 [8.36 ± 0.05]
MG 624			0.39 [6.41 ± 0.07]
DHβE			~100 [~4.0][c]
1	>100 [<4.0]		>100 [<4.0]
2a	9.0 [5.04 ± 0.03]	83 ± 4	
2b	weak agonist[b]		
2c	>100 [<4.0]		>100 [<4.0]
2d	>100 [<4.0]		>100 [<4.0]
2e			2.0 [5.69 ± 0.07]
3a			~30 [~4.5]
3b			~30 [~4.5]
3c	>100 [<4.0]		>100 [<4.0]
3d	>100 [<4.0]		>100 [<4.0]
3e	>100 [<4.0]		>100 [<4.0]
3f	>100 [<4.0]		>100 [<4.0]
4a	agonist (biphasic)[d]		
4b	agonist (biphasic)[d]		
4c	2.6 [5.58 ± 0.05]	84 ± 4	
4d	weak agonist[a]		
4e	0.99 [6.01 ± 0.12]	91 ± 3	
4f	4.4 [5.36 ± 0.09]	96 ± 3	
5a			5.7 [5.24 ± 0.07]

5b			11 [4.94 ± 0.09]
5c	weak agonist[b]		
(*R*)-**5c**	weak agonist[a]		
(*S*)-**5c**	weak agonist[b]		
5d	>100 [<4.0]		>100 [<4.0]
(*R*)-**5d**	>100 [<4.0]		>100 [<4.0]
(*S*)-**5d**	>100 [<4.0]		>100 [<4.0]
5e	weak agonist[b]		
5f	>100 [<4.0]		>100 [<4.0]
5g	>100 [<4.0]		~100 [~4.0][c]
6a	agonist (biphasic)[d]		
6b	agonist (biphasic)[d]		
6c	2.6 [5.59 ± 0.04]	80 ± 3	
(*R*)-**6c**	1.6 [5.81 ± 0.04]	85 ± 6	
(*S*)-**6c**	5.4 [5.27 ± 0.07]	78 ± 5	
6d	7.5 [5.12 ± 0.04]	59 ± 4	
(*R*)-**6d**	5.1 [5.29 ± 0.07]	78 ± 6	
(*S*)-**6d**	6.5 [5.18 ± 0.10]	79 ± 6	
6e	1.2 [5.90 ± 0.08]	84 ± 3	
6f	5.2 [5.28 ± 0.08]	81 ± 5	
6g	2.2 [5.66 ± 0.07]	82 ± 5	

[a,b] Agonist-concentration response curves not complete within the tested concentration range. Significant agonist responses observed at concentrations of 10 μM[a] or 30 μM[b].

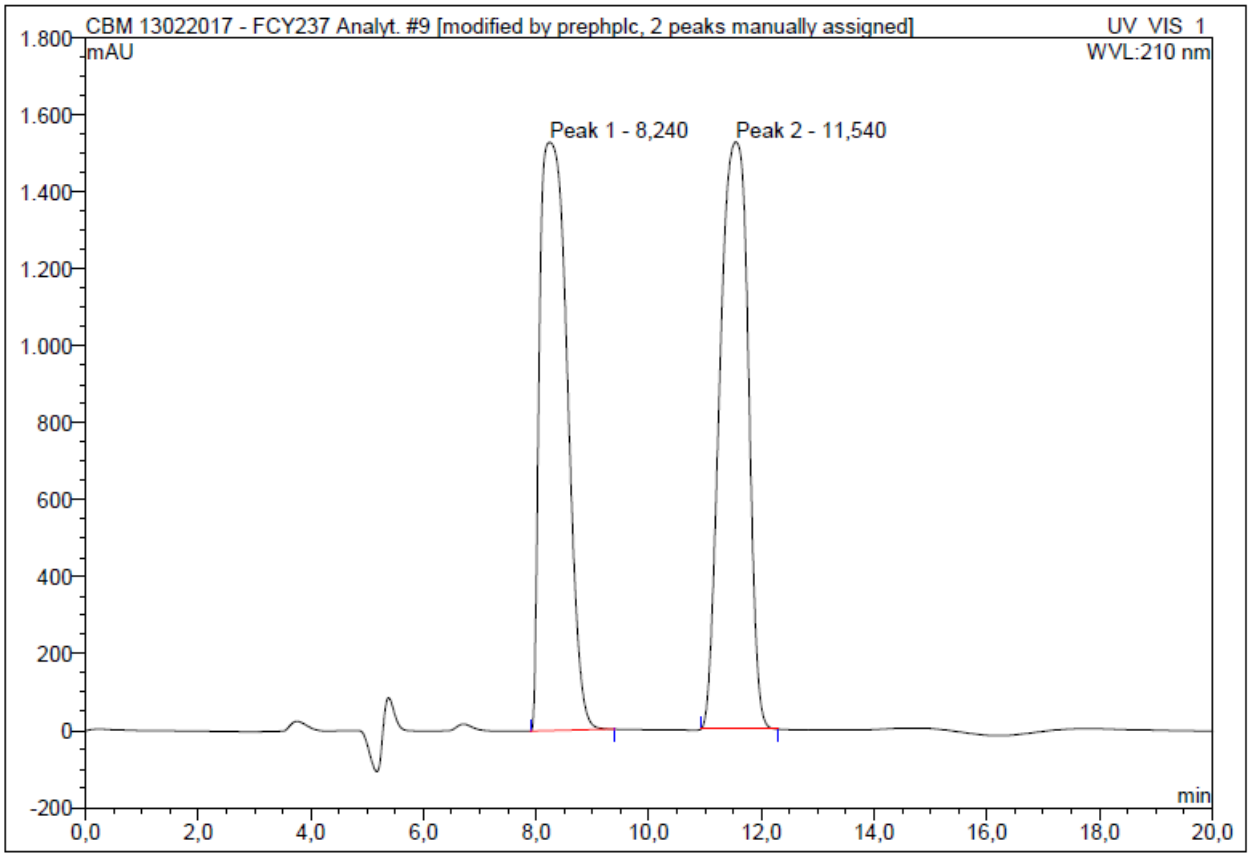
[c] The concentration-inhibition curve was not complete within the concentration range tested. Thus, the IC_{50} value was estimated from the fitted curve.

[d] Agonist-concentration response curves were biphasic. The compounds elicited significant and concentration-dependent agonist responses at lower concentrations, whereas higher concentrations elicited smaller responses. Significant agonist responses that increased with increasing concentrations were observed for 1 and 3 μM (compound **4a**) and for 0.3, 1 and 3 μM (compounds **4b**, **6a** and **6b**). At 10 μM and higher concentrations, the agonist-induced responses decreased substantially.

7 Chiral separation and HPLC chromatograms

HPLC chromatogram of racemic **5c**

Sample Name:	**FCY.237 Racemic**	*Injection Volume:*	**20,0**
Vial Number:	**8**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**210.0**
Control Program:	**CBM analytical isocratic 20 min**	*Bandwidth:*	**4**
Quantif. Method:	**CBM01**	*Dilution Factor:*	**1,0000**
Recording Time:	**13-2-2017 15:12**	*Sample Weight:*	**1,0000**
Run Time (min):	**20,00**	*Sample Amount:*	**1,0000**

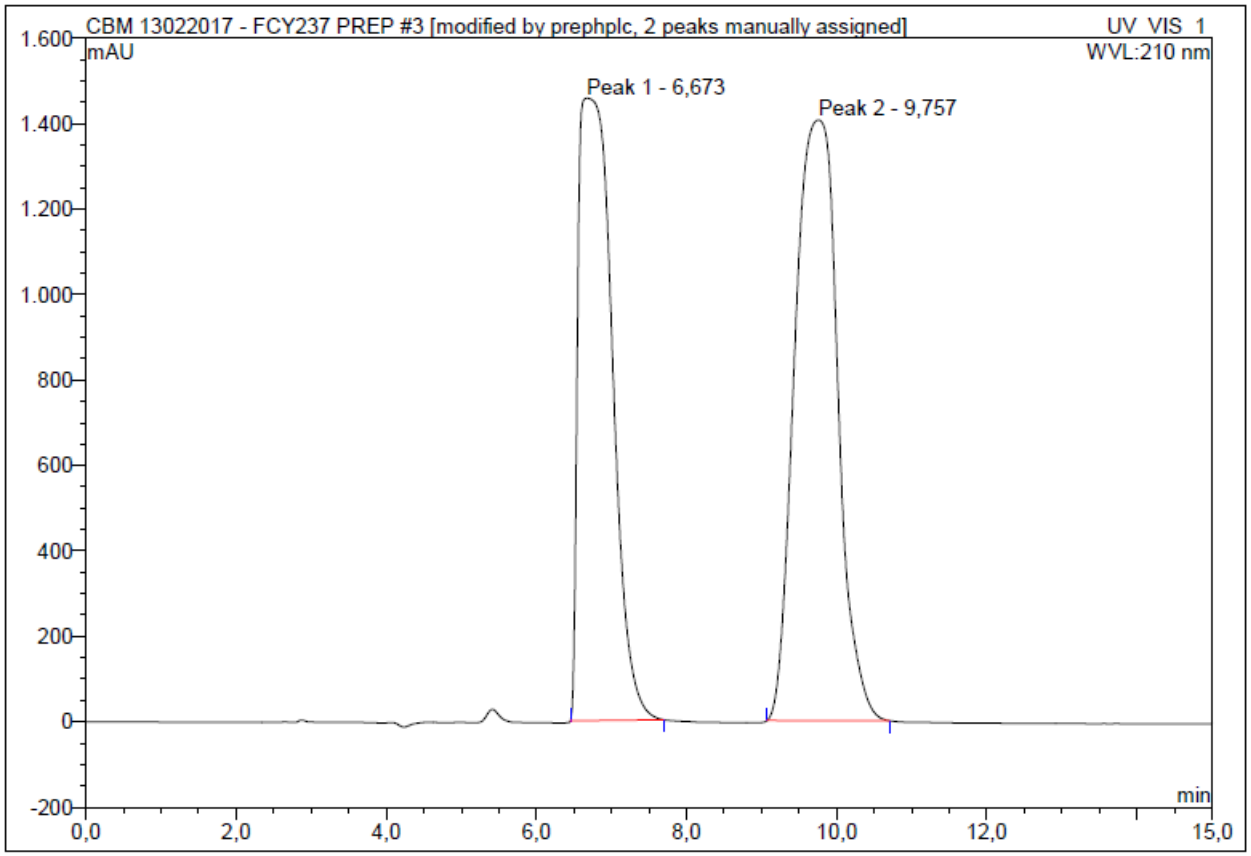


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	8,24	Peak 1	1529,264	862,853	49,45	n.a.	BMB*^
2	11,54	Peak 2	1526,710	881,987	50,55	n.a.	BMB*^
Total:			3055,974	1744,840	100,00	0,000	

Above is a representative analytical HPLC experiment on racemic **5c**. The retention time (t_R) of peak 1 was 8.24 min while the retention time (t_R) of peak 2 was 11.54 min.

Chiral HPLC separation of racemic **5c**

Sample Name:	**FCY237 20 mg/ml**	*Injection Volume:*	**2000,0**
Vial Number:	**4**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**210.0**
Control Program:	**CBM prep isocratic 95_5_15min_specified_**	*Bandwidth:*	**4**
Quantif. Method:	**CBM01**	*Dilution Factor:*	**1,0000**
Recording Time:	**13-2-2017 9:21**	*Sample Weight:*	**1,0000**
Run Time (min):	**15,00**	*Sample Amount:*	**1,0000**

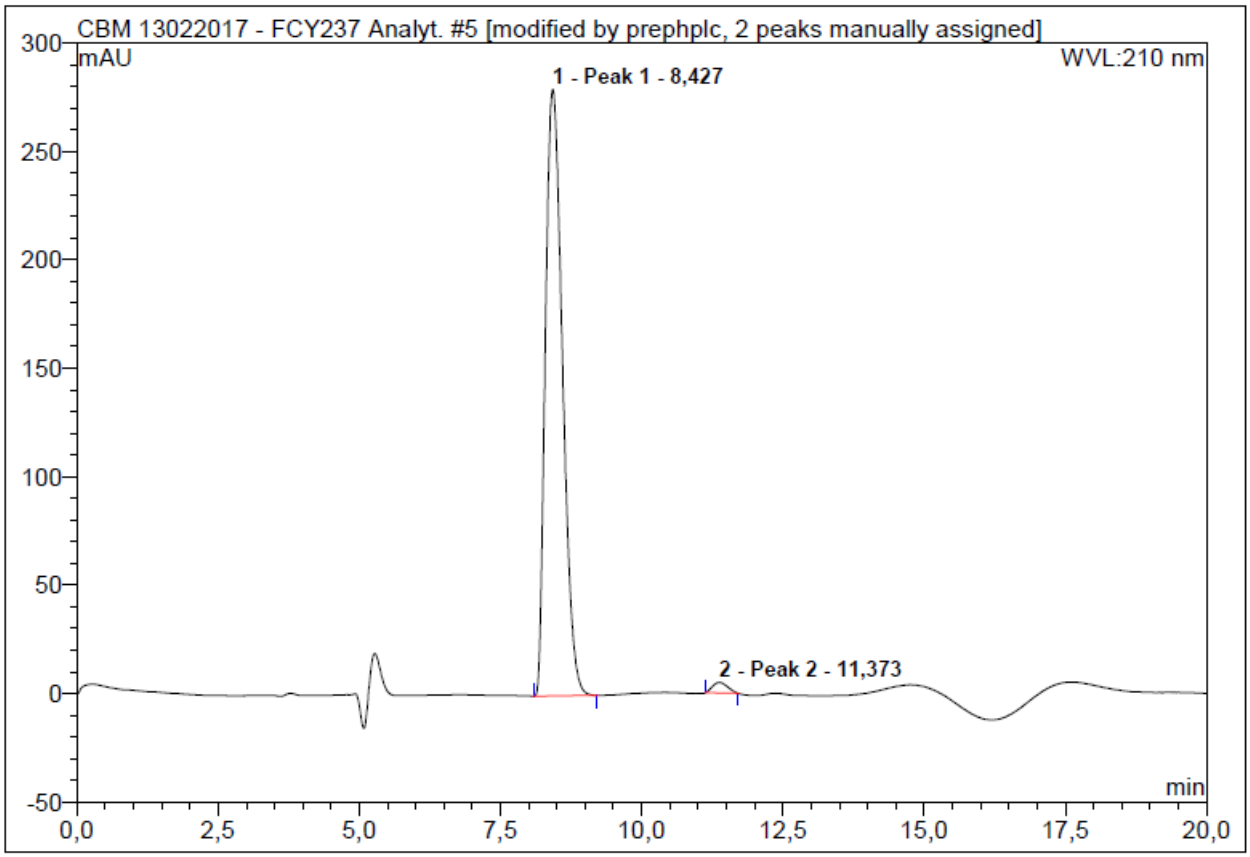


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	6,67	Peak 1	1456,735	763,777	45,03	n.a.	BMB*^
2	9,76	Peak 2	1405,961	932,378	54,97	n.a.	BMB*^
Total:			2862,696	1696,154	100,00	0,000	

Racemic **5c** (408 mg) was dissolved after sonication for 5 min in a heptane-isopropanol-diethylamine (95:5:0.1) mixture. Above is a representative example of one injection (20 mg/mL) on the preparative HPLC. All fractions containing peak 1 were collected and concentrated providing an off-white solid (172.7 mg). All fractions containing peak 2 were collected and concentrated providing an off-white solid (175.5 mg). The retention time (t_R) of peak 1 was 6.67 min while the retention time (t_R) of peak 2 was 9.76 min. These two obtained compounds were tested again on the analytical HPLC for evaluating ee and purity.

HPLC chromatogram of peak 1 after chiral HPLC separation

Sample Name:	**FCY.237 Peak 1**	*Injection Volume:*	**20,0**
Vial Number:	**7**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**210.0**
Control Program:	**CBM analytical isocratic 20 min**	*Bandwidth:*	**4**
Quantif. Method:	**CBM01**	*Dilution Factor:*	**1,0000**
Recording Time:	**13-2-2017 13:55**	*Sample Weight:*	**1,0000**
Run Time (min):	**20,00**	*Sample Amount:*	**1,0000**

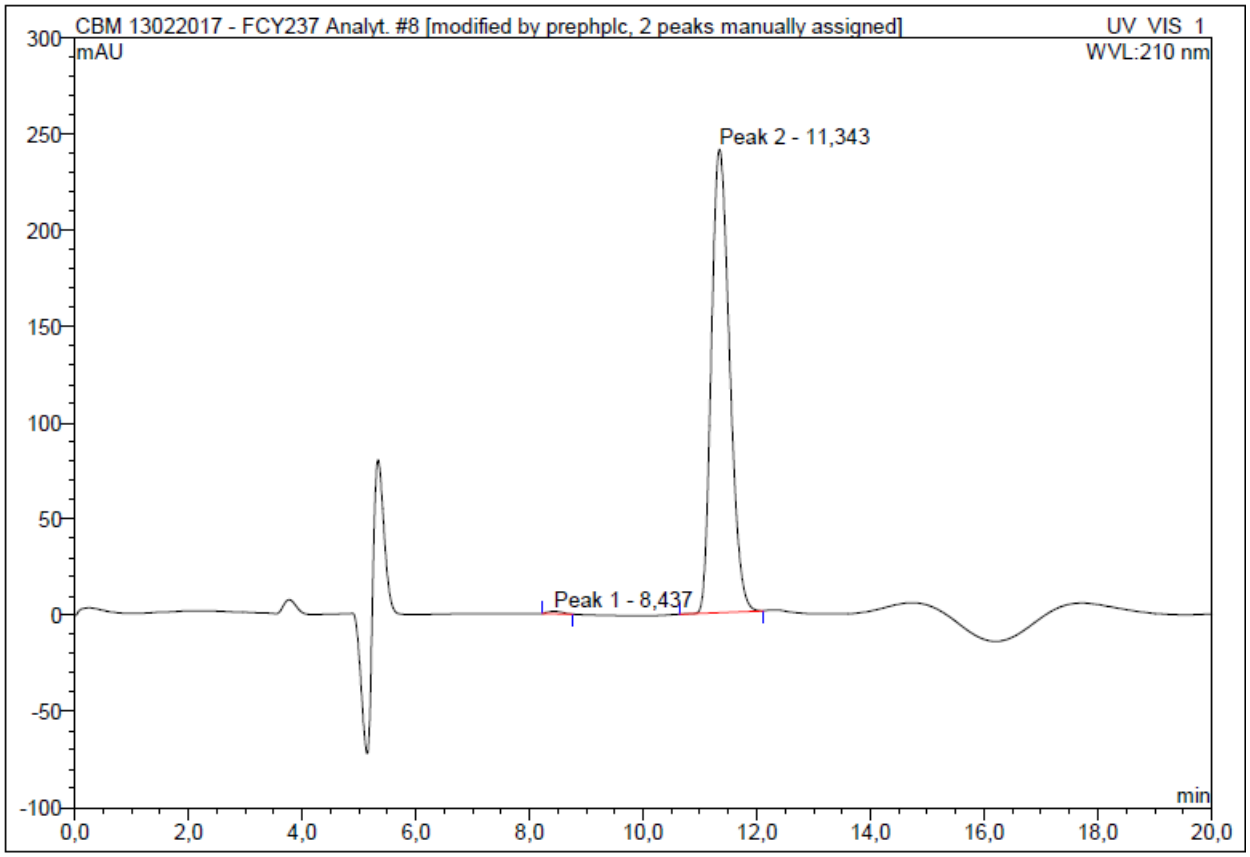


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	8,43	Peak 1	279,659	97,873	98,50	n.a.	BMB*^
2	11,37	Peak 2	4,786	1,495	1,50	n.a.	BMB*^
Total:			284,445	99,368	100,00	0,000	

Above is a representative analytical HPLC experiment on peak 1 obtained after the HPLC chiral separation. The retention time (t_R) of peak 1 was 8.43 min. The calculated ee was 97.0%.

HPLC chromatogram of peak 2 after chiral HPLC separation

Sample Name:	**FCY.237 Peak 2**	*Injection Volume:*	**20,0**
Vial Number:	**8**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**210.0**
Control Program:	**CBM analytical isocratic 20 min**	*Bandwidth:*	**4**
Quantif. Method:	**CBM01**	*Dilution Factor:*	**1,0000**
Recording Time:	**13-2-2017 14:51**	*Sample Weight:*	**1,0000**
Run Time (min):	**20,00**	*Sample Amount:*	**1,0000**



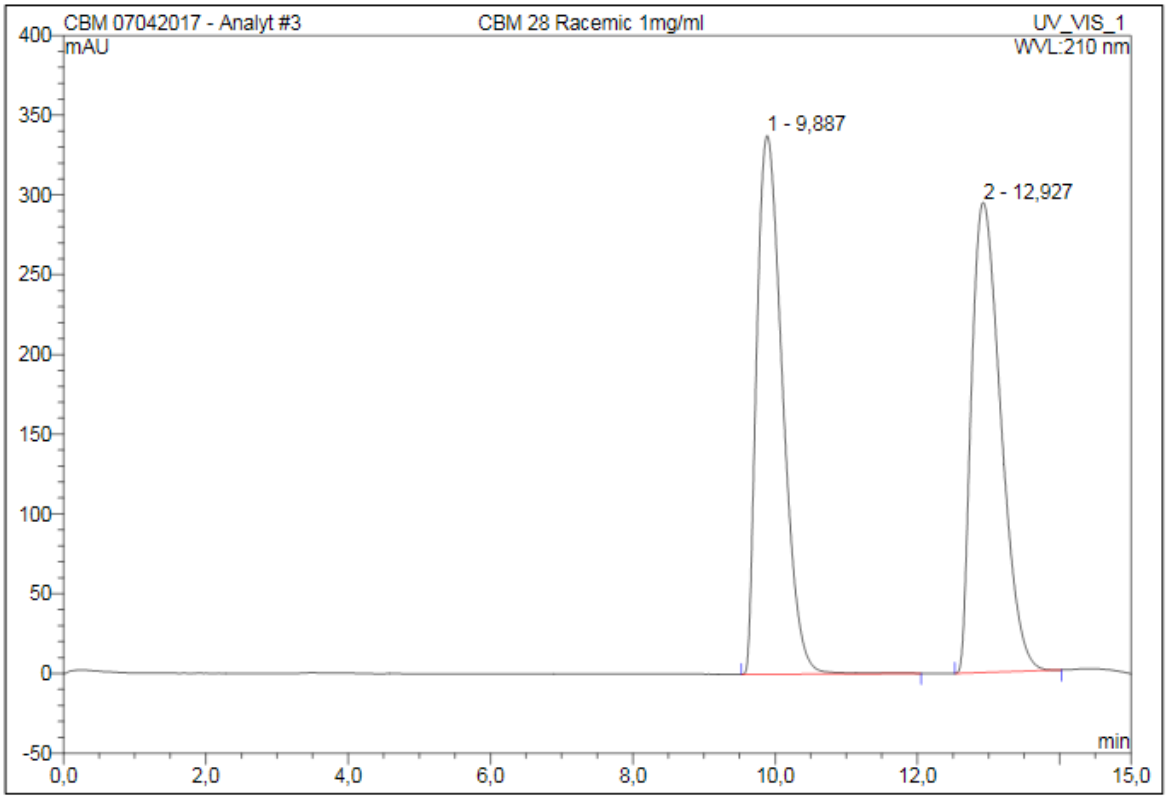
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	8,44	Peak 1	1,382	0,396	0,44	n.a.	BMB*^
2	11,34	Peak 2	240,956	89,514	99,56	n.a.	BMB*^
Total:			242,337	89,910	100,00	0,000	

Above is a representative analytical HPLC experiment on peak 2 obtained after the HPLC chiral separation. The retention time (t_R) of peak 2 was 11.34 min. The calculated ee was 99.1%.

HPLC chromatogram of racemic **5d**

3 CBM 28 Racemic 1mg/ml

Sample Name:	**CBM 28 Racemic 1mg/ml**	*Injection Volume:*	**5,0**
Vial Number:	**7**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**210.0**
Control Program:	**CBM analytical isocratic 15 min_90_10**	*Bandwidth:*	**4**
Quantif. Method:	**CBM01**	*Dilution Factor:*	**1,0000**
Recording Time:	**7-4-2017 18:16**	*Sample Weight:*	**1,0000**
Run Time (min):	**15,00**	*Sample Amount:*	**1,0000**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	9,89	n.a.	337,778	141,327	50,20	n.a.	BMB
2	12,93	n.a.	294,708	140,189	49,80	n.a.	BMB
Total:			632,486	281,516	100,00	0,000	

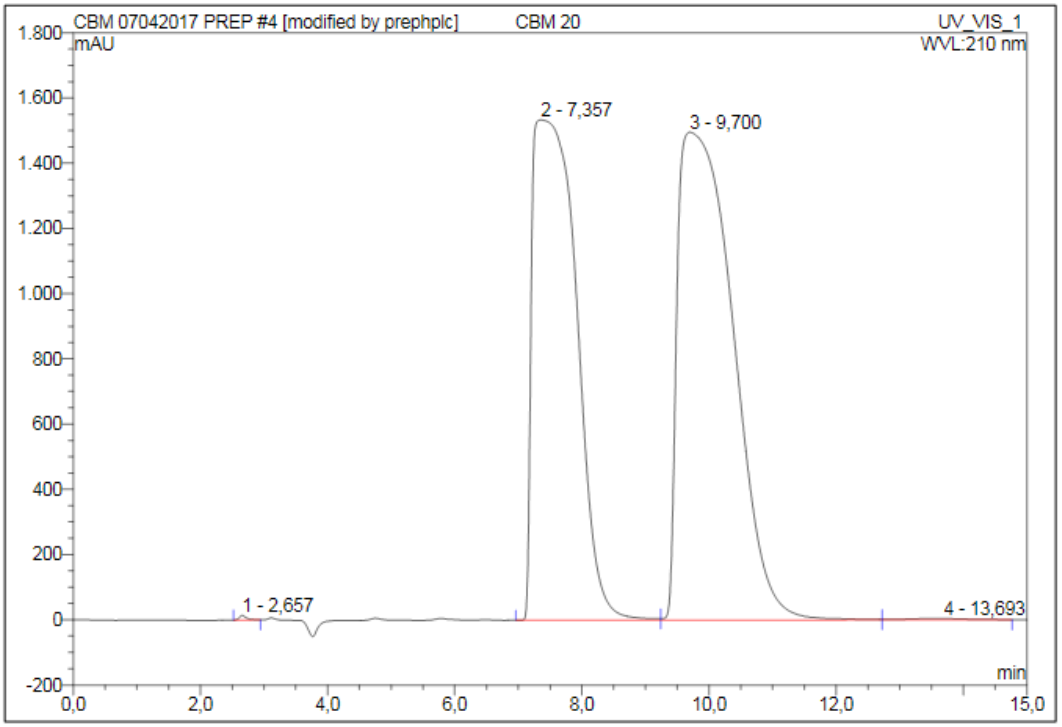
Above is a representative analytical HPLC experiment on racemic **5d**. The retention time (t_R) of peak 1 was 9.89 min while the retention time (t_R) of peak 2 was 12.93 min.

Chiral HPLC separation of racemic **5d**

4 CBM 20

3 ml og 8 mg/ml load

Sample Name:	**CBM 20**	*Injection Volume:*	**20,0**
Vial Number:	**5**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**210.0**
Control Program:	**CBM prep isocratic 90_10_15min_specifiec**	*Bandwidth:*	**4**
Quantif. Method:	**CBM01**	*Dilution Factor:*	**1,0000**
Recording Time:	**7-4-2017 13:31**	*Sample Weight:*	**1,0000**
Run Time (min):	**15,00**	*Sample Amount:*	**1,0000**



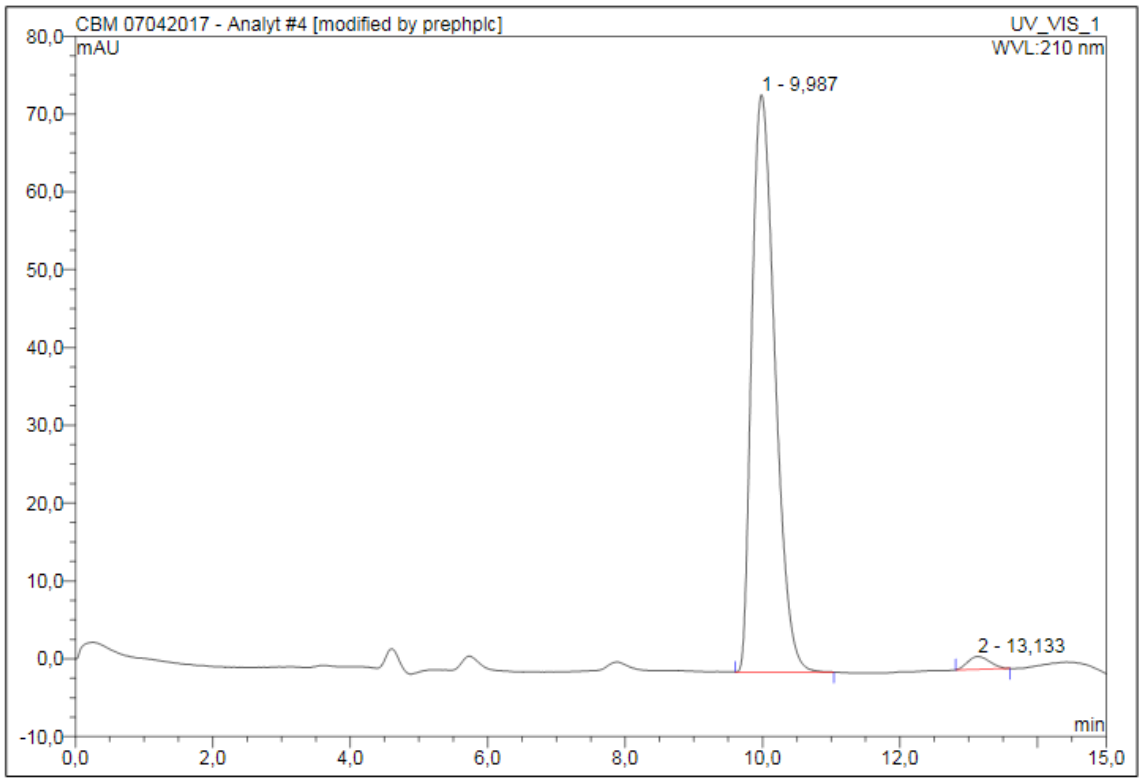
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	2,66	n.a.	14,002	1,752	0,06	n.a.	BMB*
2	7,36	n.a.	1533,836	1234,282	44,90	n.a.	BM *
3	9,70	n.a.	1495,804	1508,132	54,86	n.a.	M
4	13,69	n.a.	3,796	4,681	0,17	n.a.	MB
Total:			3047,438	2748,847	100,00	0,000	

Racemic **5d** (433 mg) was dissolved after sonication for 5 min in a heptane-isopropanol-diethylamine (90:10:0.1) mixture. Above is a representative example of one injection (8 mg/mL) on the preparative HPLC. All fractions containing peak 1 were collected and concentrated providing a white solid (140 mg). All fractions containing peak 2 were collected and concentrated providing a white solid (135 mg). The retention time (t_R) of peak 1 was 7.36 min while the retention time (t_R) of peak 2 was 9.70 min. These two obtained compounds were tested again on the analytical HPLC for evaluating ee and purity.

HPLC chromatogram of peak 1 after chiral HPLC separation

4 CBM 28 Peak I 1 mg/ml

Sample Name:	**CBM 28 Peak I 1 mg/ml**	*Injection Volume:*	**5,0**
Vial Number:	**8**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**210.0**
Control Program:	**CBM analytical isocratic 15 min_90_10**	*Bandwidth:*	**4**
Quantif. Method:	**CBM01**	*Dilution Factor:*	**1,0000**
Recording Time:	**7-4-2017 18:34**	*Sample Weight:*	**1,0000**
Run Time (min):	**15,00**	*Sample Amount:*	**1,0000**



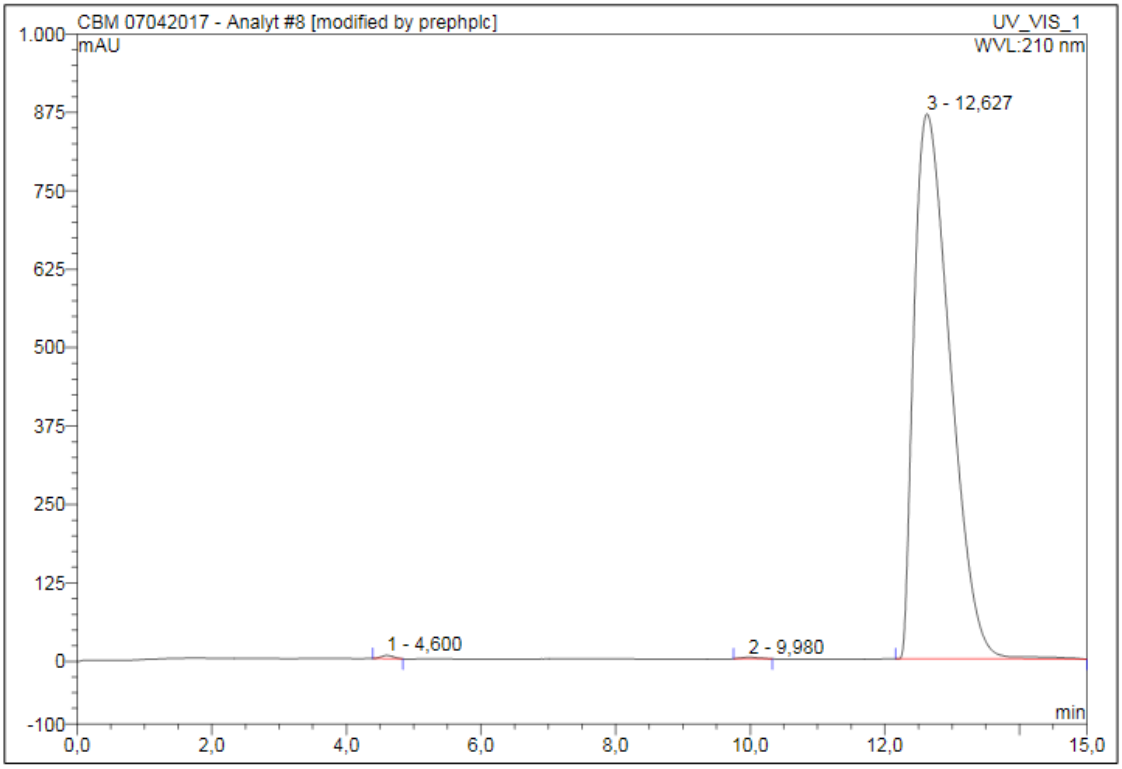
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	9,99	n.a.	74,195	28,961	97,93	n.a.	BMB
2	13,13	n.a.	1,643	0,611	2,07	n.a.	BMB*
Total:			75,838	29,572	100,00	0,000	

Above is a representative analytical HPLC experiment on peak 1 obtained after the HPLC chiral separation. The retention time (t_R) of peak 1 was 9.99 min. The calculated ee was 95.9%.

HPLC chromatogram of peak 2 after chiral HPLC separation

8 CBM 20 Peak II 1 mg/ml

Sample Name:	**CBM 20 Peak II 1 mg/ml**	*Injection Volume:*	**5,0**
Vial Number:	**11**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**210.0**
Control Program:	**CBM analytical isocratic 15 min_90_10**	*Bandwidth:*	**4**
Quantif. Method:	**CBM01**	*Dilution Factor:*	**1,0000**
Recording Time:	**7-4-2017 19:36**	*Sample Weight:*	**1,0000**
Run Time (min):	**15,00**	*Sample Amount:*	**1,0000**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	4,60	n.a.	4,998	1,094	0,20	n.a.	BMB*
2	9,98	n.a.	2,390	0,748	0,14	n.a.	BMB*
3	12,63	n.a.	868,675	541,412	99,66	n.a.	BMB
Total:			876,064	543,253	100,00	0,000	

Above is a representative analytical HPLC experiment on peak 2 obtained after the HPLC chiral separation. The retention time (t_R) of peak 2 was 12.63 min. The calculated ee was 99.3%.

8 Optical rotation data

An analytical sample of (*R*)-**5c** (after HPLC separation with a 99.1% ee) was obtained for calculation of its optical rotation at 23.7 °C.

$[\alpha]_D$ = + 120.0 (*c* = 1.63, chloroform).

An analytical sample of (*S*)-**5c** (after HPLC separation with a 97.0% ee) was obtained for calculation of its optical rotation at 23.7 °C.

$[\alpha]_D$ = – 125.2 (*c* = 1.61, chloroform).

An analytical sample of (*R*)-**5d** (after HPLC separation with a 99.3% ee) was obtained for calculation of its optical rotation at 25.2 °C.

$[\alpha]_D$ = + 111.7 (*c* = 0.43, chloroform). Lit $[\alpha]_D$ = + 100.4 ° (*c* = 1.0, chloroform).[3]

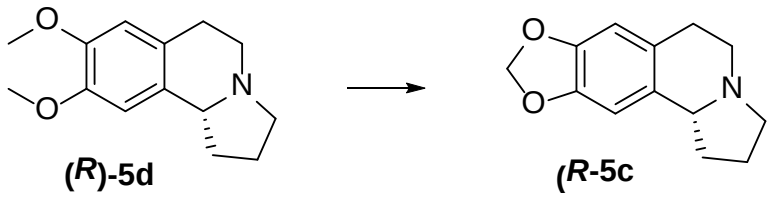
An analytical sample of (*S*)-**5d** (after HPLC separation with a 95.9% ee) was obtained for calculation of its optical rotation at 25.3 °C.

$[\alpha]_D$ = – 109.7 (*c* = 0.49, chloroform). Lit $[\alpha]_D$ = – 100.1 ° (*c* = 0.32, chloroform).[4]

[3] (*R*)-**5d** is also known as (*R*)-(+)-crispine A. For more details regarding its first enantioselective synthesis and optical rotation measurement, see: Szawkało, J.; Zawadzka, A.; Wojtasiewicz, K.; Leniewski, A.; Drabowicz, J.; Czarnocki, Z. *Tetrahedron: Asymmetry* **2005**, *16*, 3619.

[4] (*S*)-**5d** is also known as (*S*)-(−)-crispine A. For more details regarding its synthesis and optical rotation measurement, see: Amat, M.; Elias, V.; Llor, N.; Subrizi, S.; Molins, E.; Bosch, J. *Eur. J. Org. Chem.*, **2010**, 4017.

9 Determination of the absolute configuration of (*R*)-5c



(*R*)-**5d** (*R*-**5c**

Synthesis: Indolizidine (*R*)-**5d** (195 mg, 0.84 mmol, 1 equiv), corresponding to the second peak obtained from the chiral separation of crispine A which is also known as (*R*)-(+)-crispine A, was dissolved in dry DCM (20 mL) and cooled to 0 °C prior to add neat BBr_3 (396 μL, 4.18 mmol, 5 equiv) dropwise. The resulting mixture was allowed to warm up to room temperature and stirred at this temperature for 2 h then cooled to 0 °C and quenched by addition of MeOH. Volatiles were removed under reduced pressure. MeOH was added again and volatiles were removed under reduced pressure. This procedure was repeated five times and furnished a crude material which was dissolved in dry DMF (3 mL). Dry potassium carbonate (577 mg, 4.18 mmol, 5 equiv) was added and the reaction mixture was stirred for 5 min at room temperature prior to add CH_2Br_2 (293 μL, 4.18 mmol, 5 equiv) dropwise. The resulting mixture was heated at 75 °C for 14 h then cooled to room temperature. After addition of water, the aqueous layer was extracted three times with EtOAc then the combined organic layers were washed three times with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to lead to crude synthetic (*R*)-**5c** as a yellowish semi-solid.

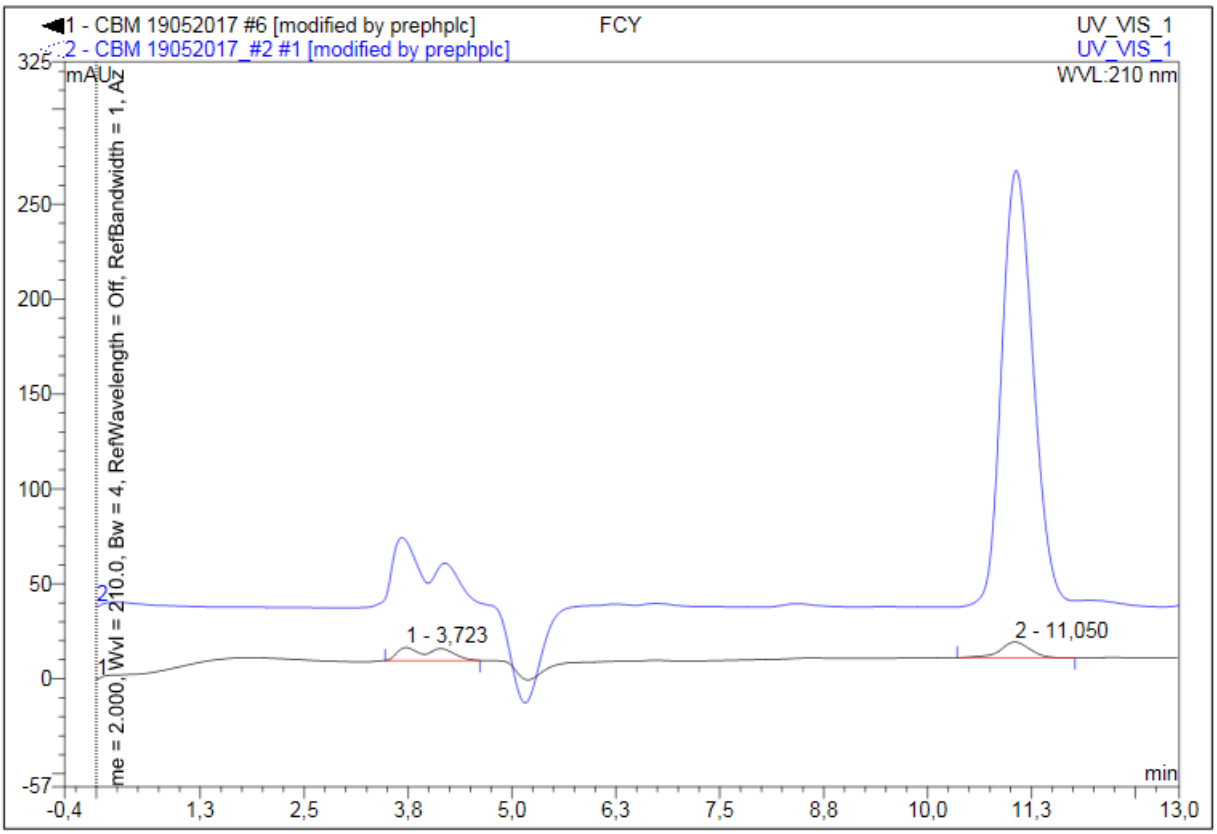
HPLC investigation: Injection of 5 μL of crude synthetic (*R*)-**5c** (5 mg/mL) dissolved in heptane-isopropanol-diethylamine (50:50:0.1) on the analytical HPLC provided the chromatogram below (black trace).

A spiking experiment was also performed where 15 μL of the second peak obtained after the chiral resolution of ligand **5c** (3 mg/mL) dissolved in heptane-isopropanol-diethylamine (50:50:0.1) and 15 μL of crude synthetic (*R*)-**5c** (5 mg/mL) dissolved in heptane-isopropanol-diethylamine (50:50:0.1) were injected together on the analytical HPLC which provided the chromatogram below (blue trace).

Thus, analytical HPLC comparisons showed that the retention time for synthetic (*R)*-**5c** was corresponding to the same one observed on the analytical HPLC chromatogram of the second peak obtained after the chiral resolution of racemic ligand **5c**. This provided a complete proof on the assignment of the absolute configuration of the two enantiomers (*S*)-**5c** and (*R*)-**5c** (and consequently (*S*)-**6c** and (*R*)-**6c**).

6 FCY

Sample Name:	**FCY**	*Injection Volume:*	**20,0**
Vial Number:	**10**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**210.0**
Control Program:	**CBM analytical isocratic 13 min**	*Bandwidth:*	**4**
Quantif. Method:	**CBM01**	*Dilution Factor:*	**1,0000**
Recording Time:	**19-5-2017 11:49**	*Sample Weight:*	**1,0000**
Run Time (min):	**13,00**	*Sample Amount:*	**1,0000**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	3,72	n.a.	6,857	4,153	53,64	n.a.	BMB*
2	11,05	n.a.	8,363	3,589	46,36	n.a.	MB*
Total:			15,220	7,742	100,00	0,000	

Analytical HPLC chromatograms of crude synthetic (*R*)-**5c** (black trace) and crude synthetic (*R*)-**5c** spiked with the second peak obtained after the chiral resolution of ligand **5c** (blue trace).

10 Computational chemistry

Docking procedure. All compounds were prepared using the LigPrep module in the Schrödinger suite to generate the three dimensional structures and to determine the ionization states using the Epik package [21]. The dockings of the investigated ligands were performed with GlideXP. The docking was performed on the X-ray structure of the human α4β2 nAChR with an additional water molecule added to the binding site as suggested by the the high resolution crystal structure 1UW6 [22]. This water molecule showed some important interactions with the compounds, increasing the correlation of determination from 0.17 to 0.47 by constraining this interaction for all the compounds. Using this constrain, a correlation of determination of 0.58 was obtained for the enantiopure derivatives. All docking runs were carried out at physiological pH (7.4) and at default settings except the van der Waals radius scaling factor which was set to 0.9 in order to allow closer contacts of poses because of the C-Loop flexibility [23].

Docking poses of several ligands. In Figure 1, the re-docking of nicotine is performed with satisfactory root mean square displacement (RMSD) of 0.66 Å indicating that the docking model is performing well. Comparing the binding mode of nicotine in the crystal structure with the conformation obtained from the docking shows that the two nitrogens are interacting with the same residues, and the overall binding mode is similar as reflected by the low RMSD. The importance of the H-bond acceptor is illustrated in Figure 2 as compound **5g** has no hydrogen acceptor moiety in its aromatic ring system close to the water molecule resulting in a poorer binding affinity.

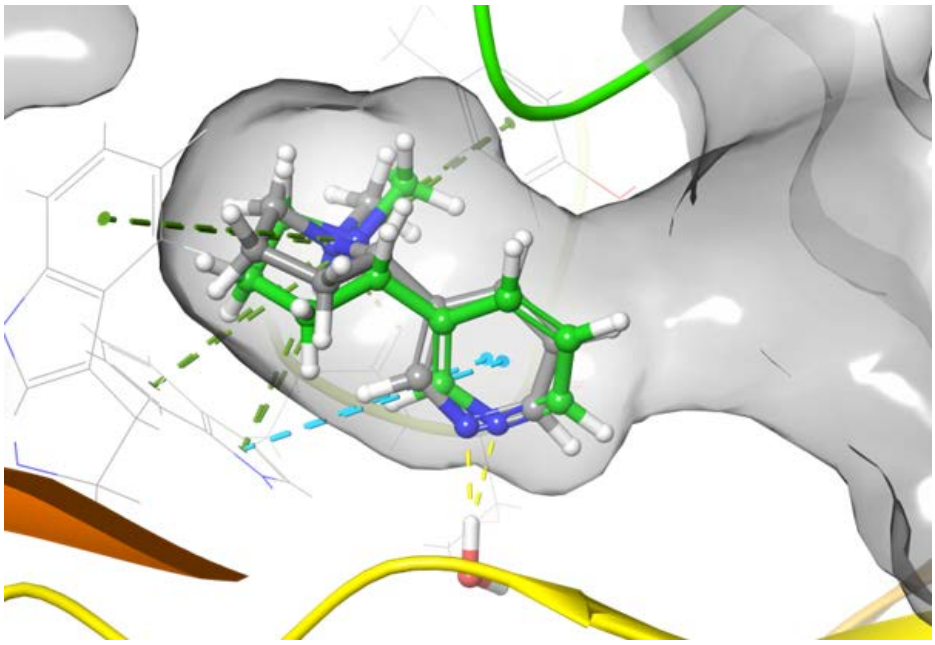


Figure 1. GlideXP re-docking of the nicotine molecule. Nicotine from PDB (PDB ID: 5KXI) in grey and docked nicotine in green. Ligand heavy atom RMSD = 0.66 Å. Hydrogen-bonds are shown in yellow dashed lines, π-cation interactions are shown in green dashed lines, and π-π stacking interactions are shown in blue dashed lines.

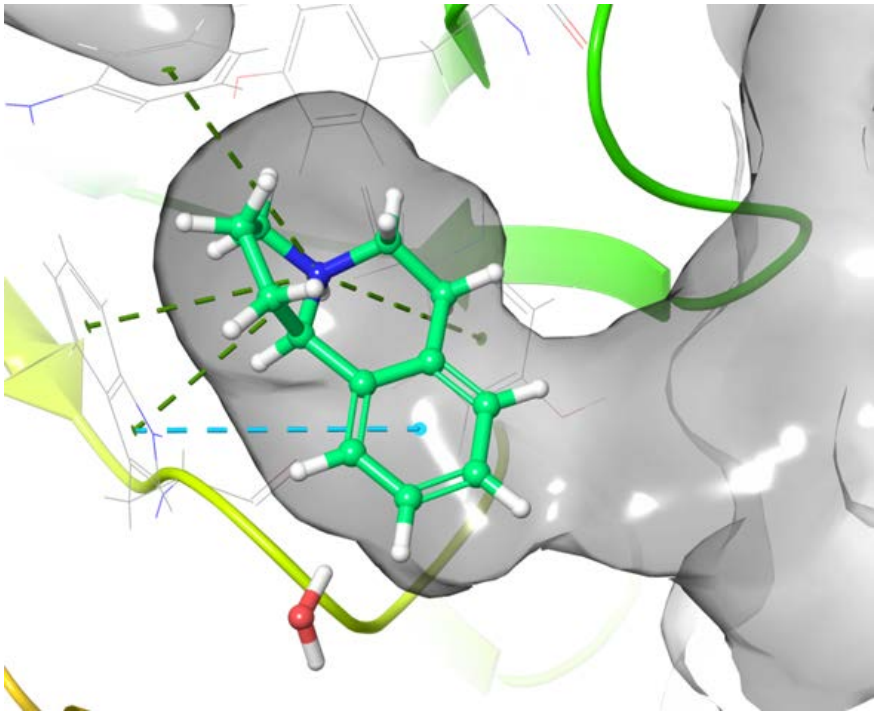


Figure 2. GlideXP docking of (*R*)-**5g**. π-cation interactions are shown in green dashed lines, and π-π stacking interaction is shown in blue dashed line.

Authors will release the atomic coordinates and experimental data upon article publication.

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